An NMR Based Similarity Metric for Higher Order Structure Assessment among U.S. Marketed Insulin Therapeutics

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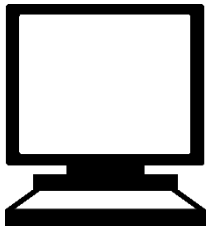
Office of Pharmaceutical Quality (OPQ)
Center for Drug Evaluation and Research (CDER)
U.S. Food and Drug Administration (FDA)

Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.


FDA
Patients expect safe and effective medicine with every dose they take.
www.fda.gov

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.


It is what gives patients confidence in their *next* dose of medicine.

Disclaimer

THIS PRESENTATION REFLECTS THE VIEWS OF THE AUTHOR AND SHOULD NOT BE CONSTRUCTED TO REPRESENT FDA'S VIEWS OR POLICES.

Agency's Guidance

A meaningful comparative analytical assessment depends on, among other things, the capabilities of available state-of-the-art analytical assays to assess, for example, the molecular weight of the protein, complexity of the protein (higher order structure and posttranslational modifications), degree of heterogeneity, functional properties, impurity profiles, and degradation profiles denoting stability.

Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations, *https://www.fda.gov/media/125484/download*

The sameness of active ingredient in a proposed generic synthetic peptide can be established through physicochemical characterization and biological evaluation. … the following properties and other properties, as appropriate: …Secondary structure; Oligomer/Aggregation states…

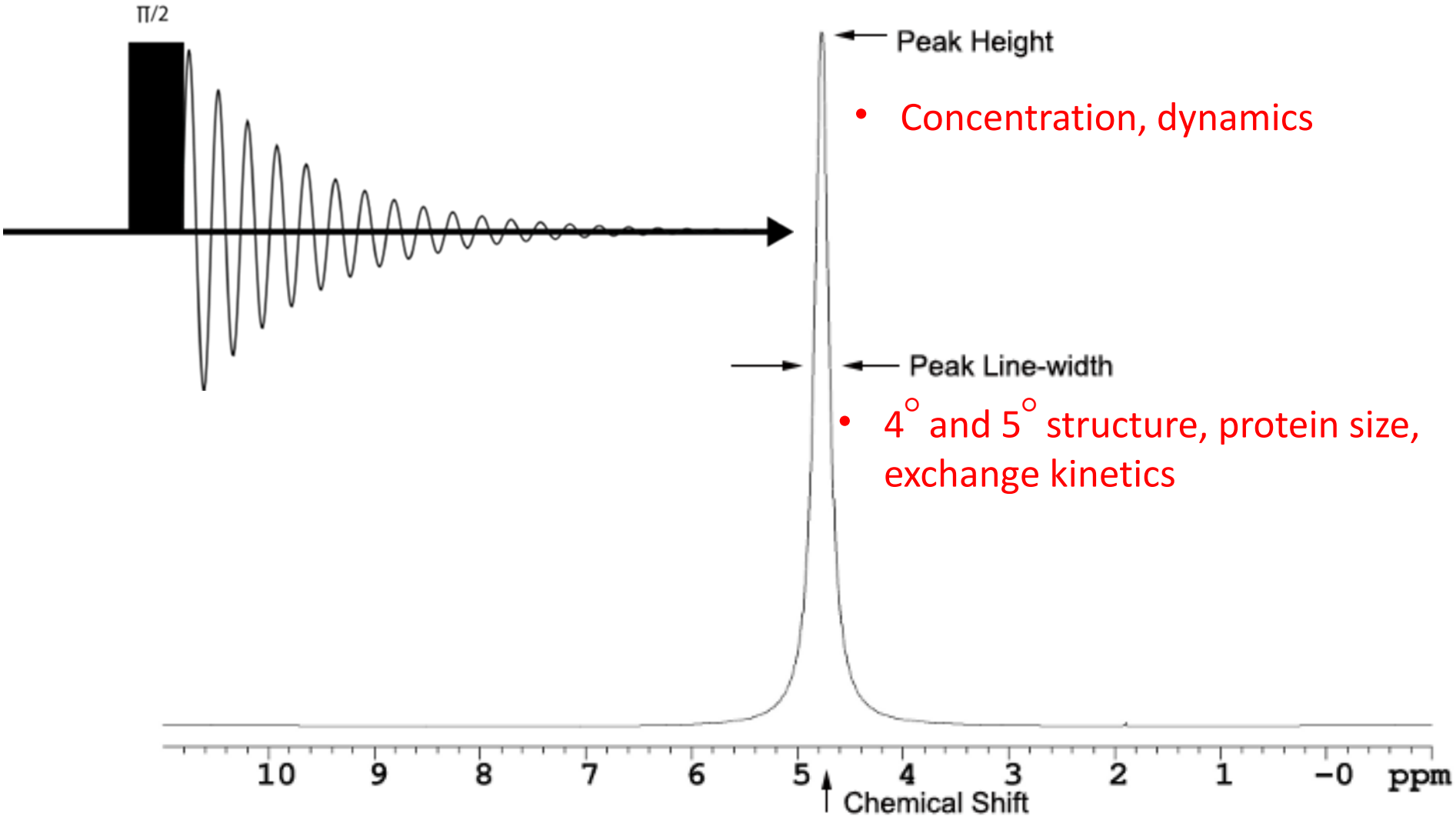
https://www.fda.gov/files/drugs/published/ANDAs-for-Certain-Highly-Purified-Synthetic-Peptide-Drug-Products-That-Refer-to-Listed-Drugs-of-rDNA-Origin-Guidance-for-Industry.pdf

Higher Order Structure

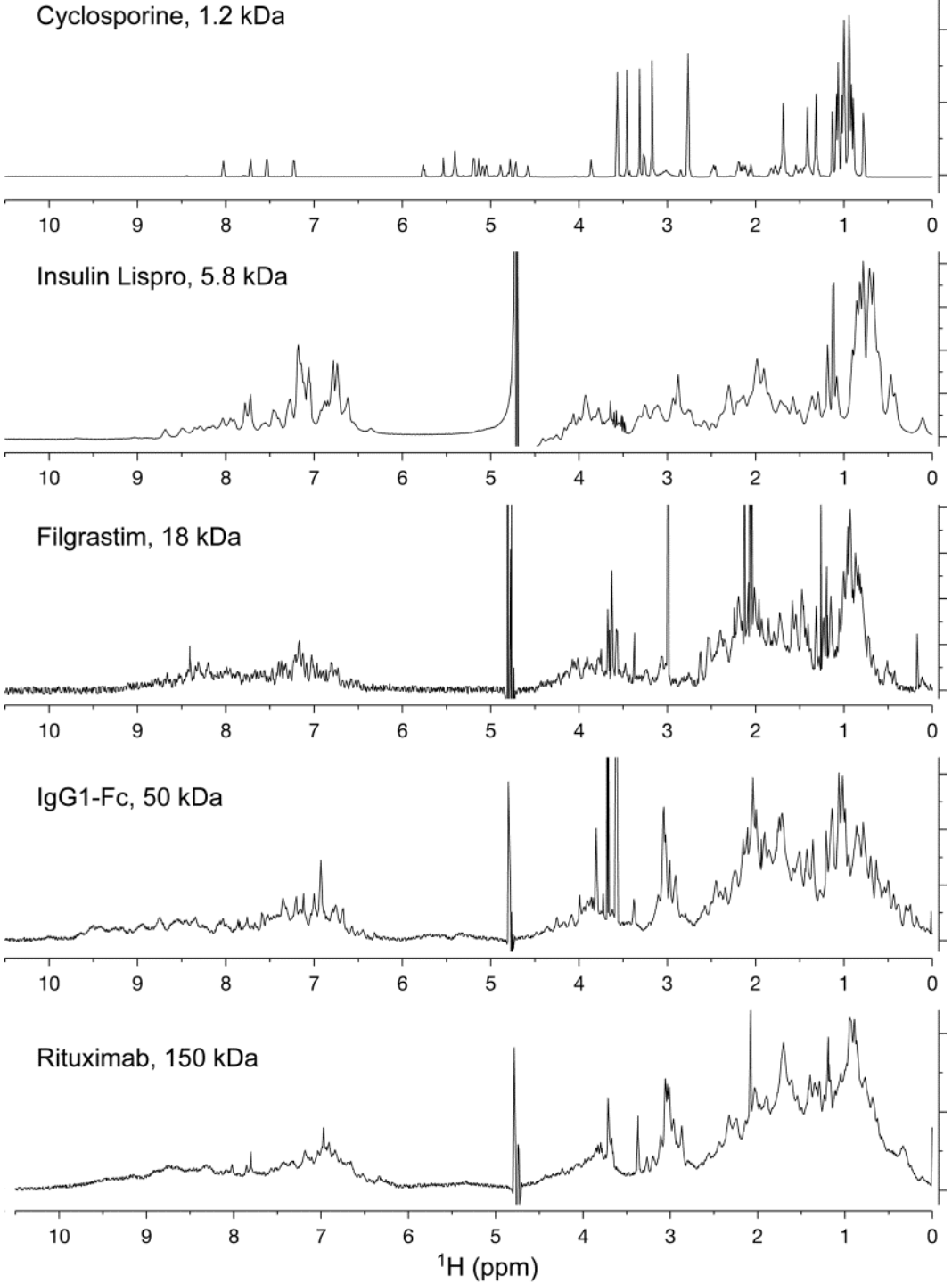
- **Standard definition**: any non-covalent interaction (e.g., H-bond) stabilized secondary, tertiary and quaternary structures.

- **Broad definition**: quinary structure, oligomerization, aggregation, equilibrium and exchange kinetics among different structural forms, e.g., between folding/unfolding, dimer/hexamer etc.

What a protein/peptide NMR peak reveals



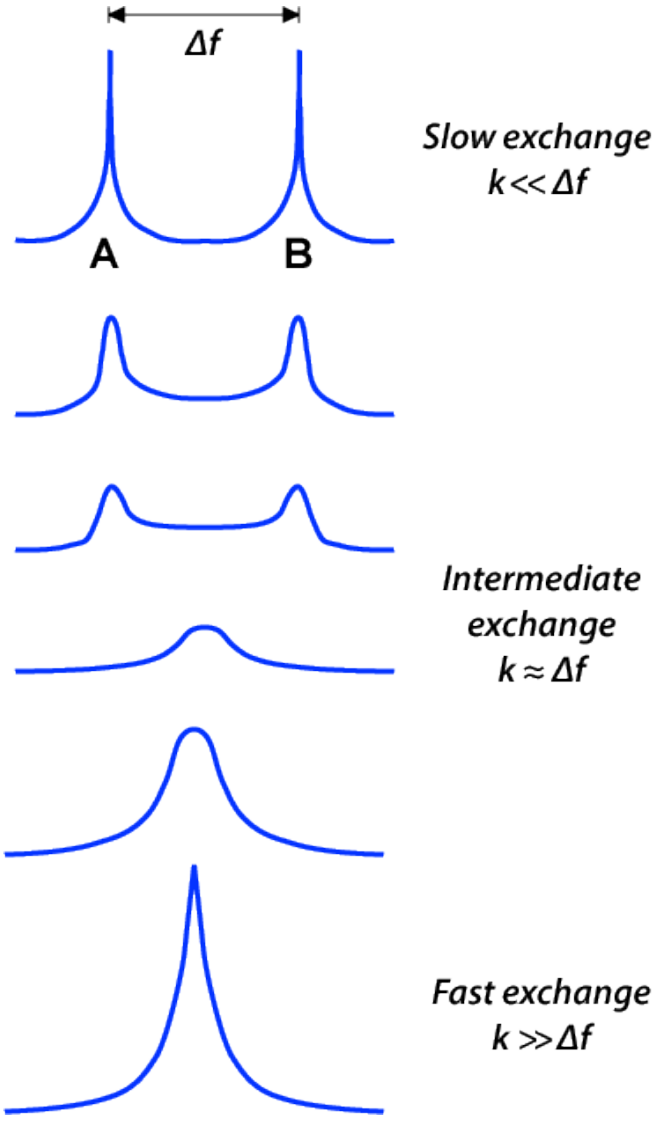
- Concentration, dynamics
- 4° and 5° structure, protein size, exchange kinetics
- Chemical identity and 2° and 3° structure



Protein and NMR spectra

Slower tumbling,
Faster relaxation,
Line broadening.

Exchange Kinetics and NMR Line-broadening



$$A \longleftrightarrow B$$

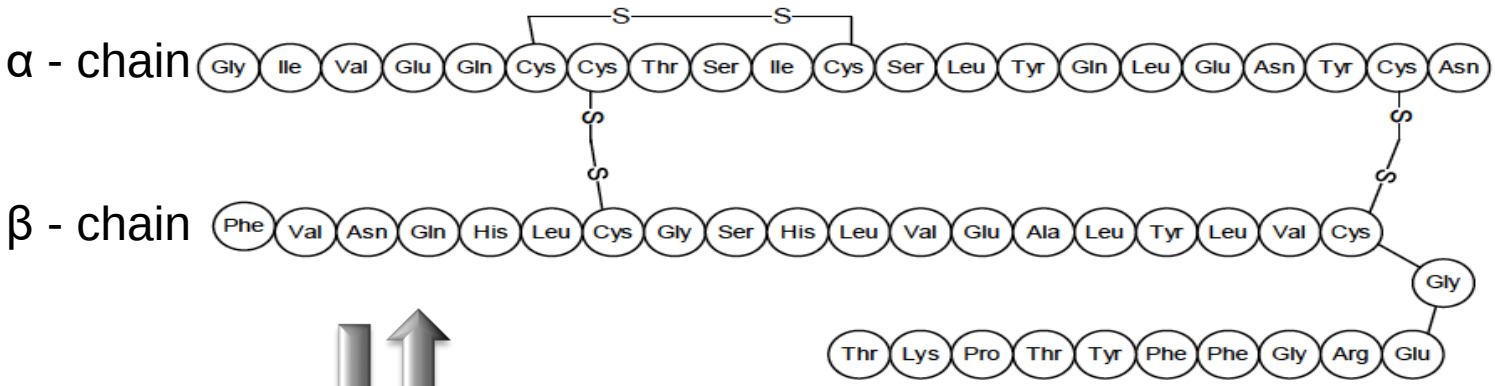
$$k=k_{AB}+k_{BA},\quad \Delta f=|f_A-f_B|$$

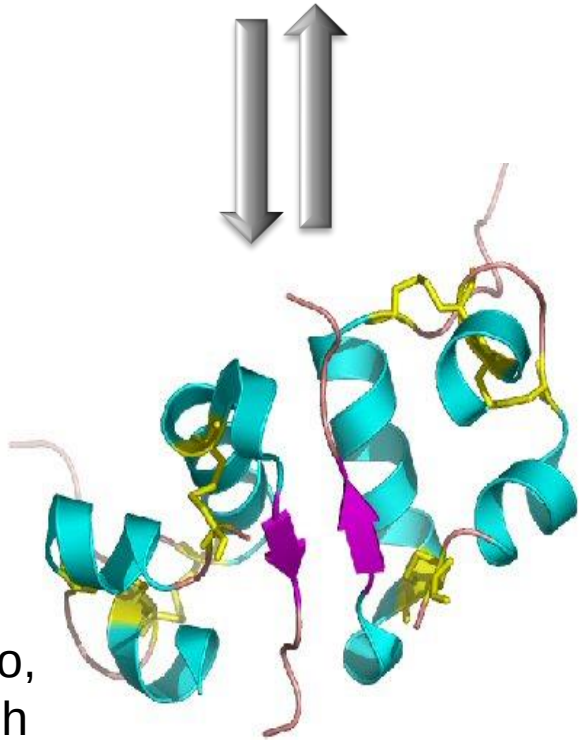
Exchange: chemical or conformation;

Exchange/heterogeneity is common for any molecule in solution.

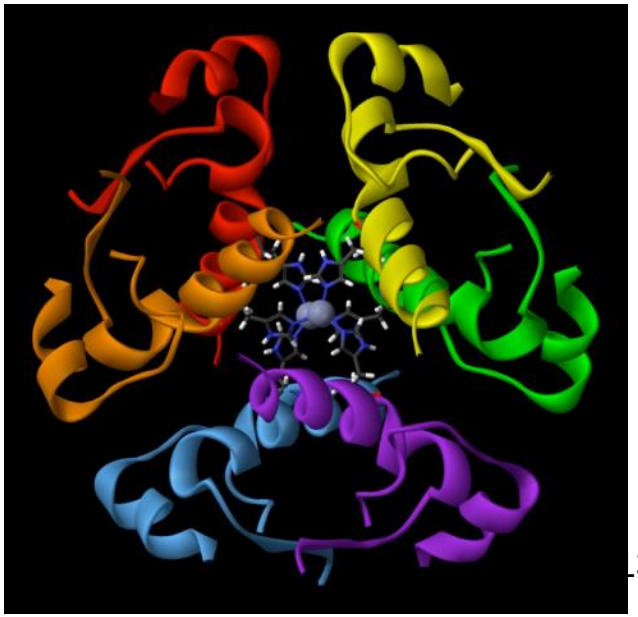
Palmer, Kroenke, and Loria. (2001) Method Enzyml.

Insulin Structure and Equilibrium





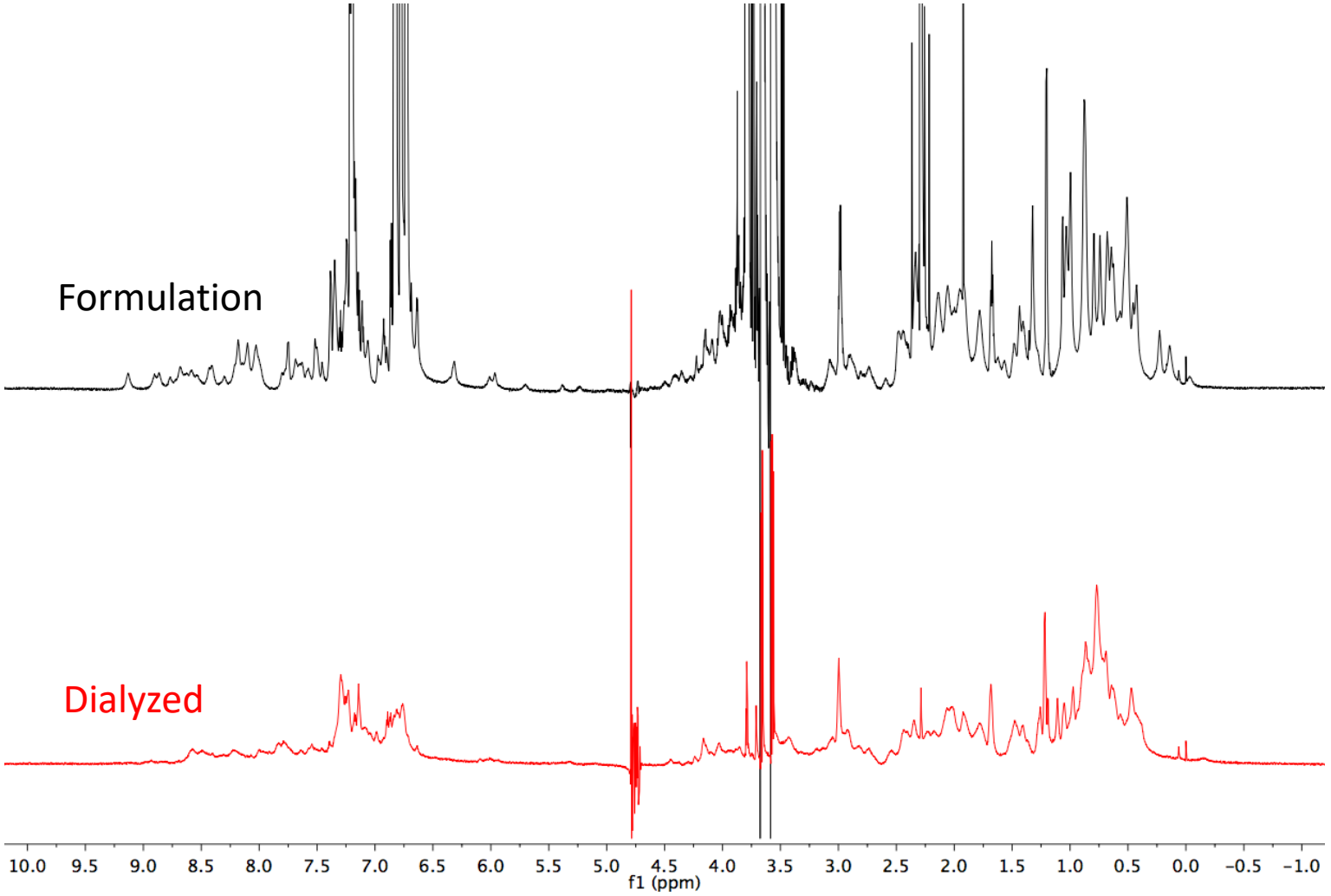
Phenol
Zn^{++}



Pdb:1mso,
1ev6, 1lph

Journal of Molecular Biology [2002, 318(2):479-490]

Insulin spectra under different buffer



Both chemical shift and line-width changed upon dialysis.

Ideal NMR Approaches for HOS Comparison

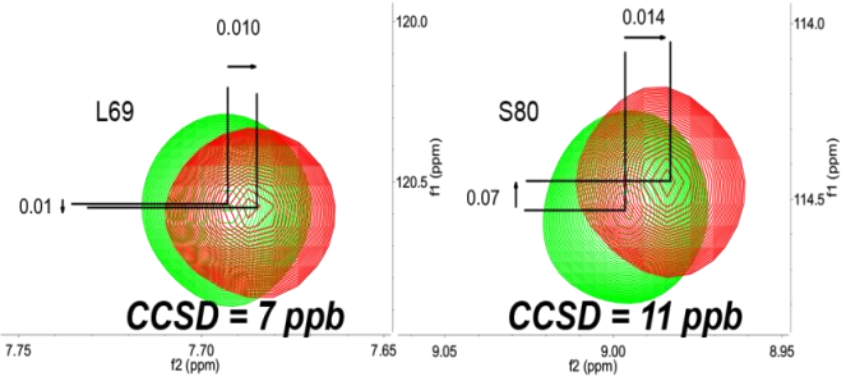
- Direct testing on drug products whenever possible b/c
  - *HOS can change upon formulation difference;*
  - *Better reproducibility;*
  - *Generic/biosimilar sponsors may not have access to DS of RLD.*

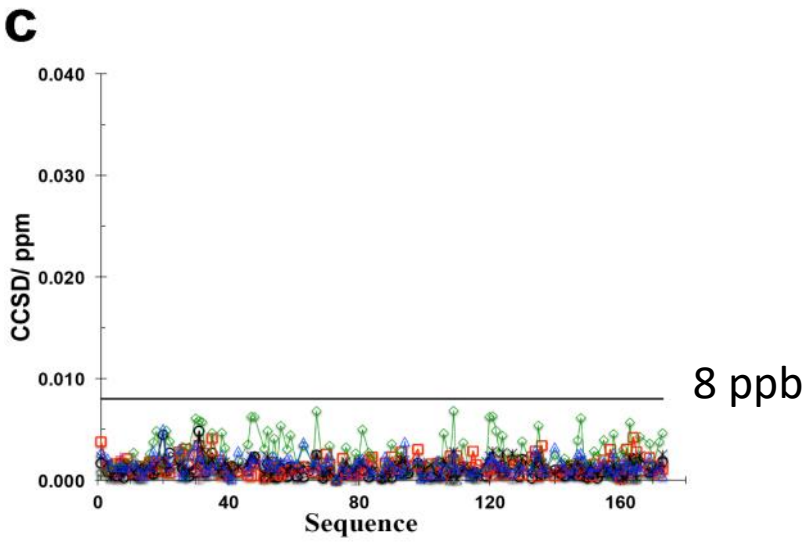
- Identical NMR conditions, *i.e*. temperature, field strength, probe, tuning and matching, pulse sequence, effective water suppression *etc*.

- Un-supervised and robust chemometric analysis to quantify NMR inter-spectra difference.

- Quantified difference to understand realistic HOS similarity.

Quantifying Chemical Shift Difference





8 ppb

CCSD=

"combined chemical shift difference"

$\sqrt{[0.5*(\delta_H^2+(\alpha * \delta_N)^2)]}$

- Sensitive;
- Need peak picking;
- No count on peak intensity;
- PCA was also performed but no proposed similarity metrics.

Ghasriani, Hodgson, Brinson, McEwen, Buhse, Kozlowski, Marino, Aubin & Keire 2016 Nature Biotechnology

Quantification of Spectral Difference

- Inter-brand similarity: **Mahalanobis distances (D_M)**
- 1D NMR with PCA may be sufficiently differentiating.

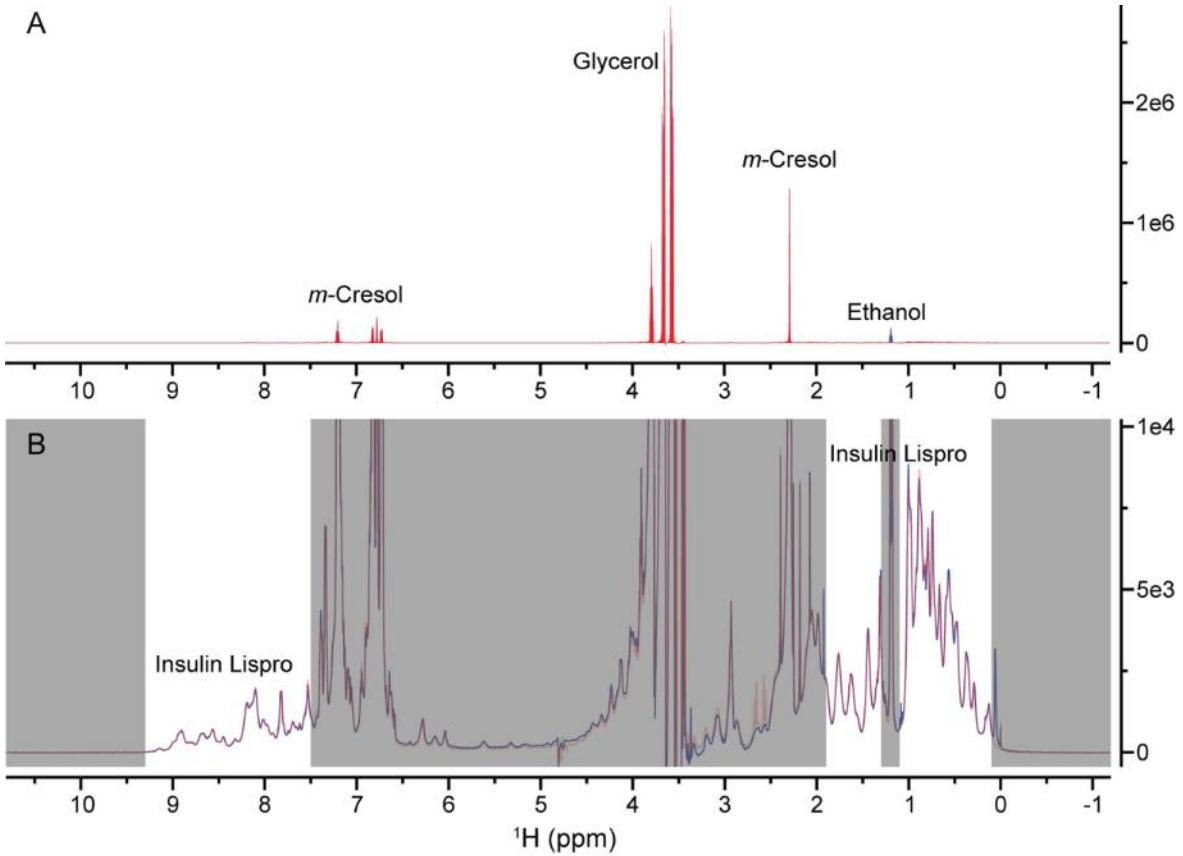
NMR spectra	Chemometric Method	Mahalanobis distances (D_M^2)
1D	PCA	213
2D	PCA	255
	Tucker3	305
	Graph Invariant (GI)	40.4

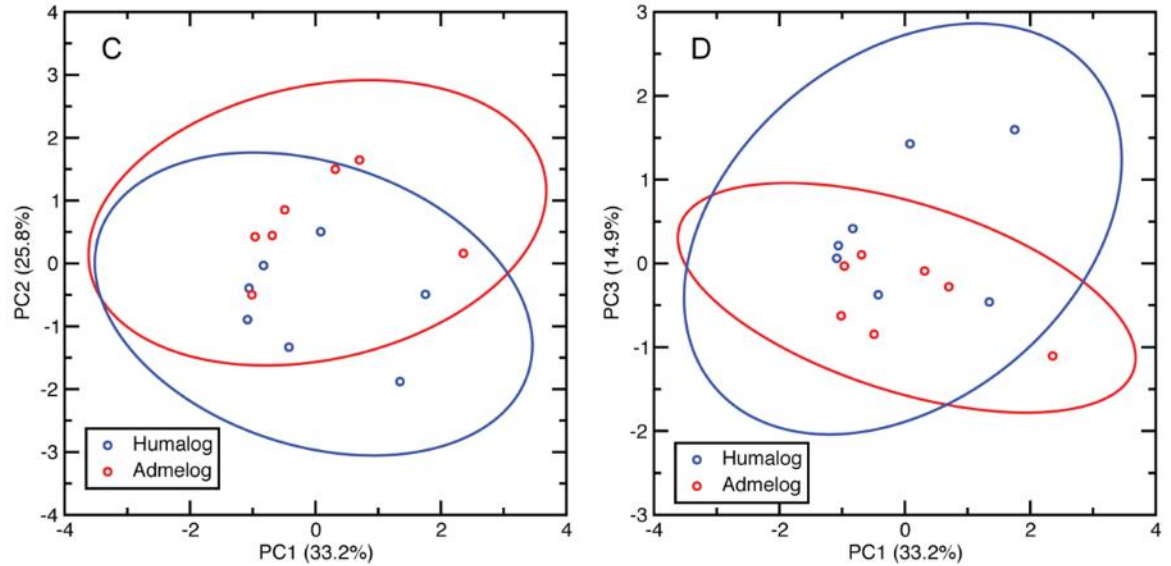
Chen, Park, Li, Patil & Keire 2017 AAPS-PST

Some of US Marketed Insulin Drug Products

Insulin Type	Drug Substance	Drug Product	Approval Type	Year approved
Rapid acting	Insulin Lispro B28: P->K B29: K->P	Humalog®	New Drug	1996
		Admelog®	Follow-on 505(b)(2)	2017
Long acting	Insulin Glargine B3: N->K B29: K->E	Lantus®	New Drug	2000
		Basaglar®	Follow-on 505(b)(2)	2015
Short acting	Insulin Human	HumulinR®	New Drug	1982
		NovolinR®	New Drug	1991

Wang, D.; Park; Patil, S.; Smith, C.; Leaser, J.; Keire, D.; Chen, K., An NMR Based Similarity Metric for Higher Order Structure Quality Assessment among U.S. Marketed Insulin Therapeutics. J. Pharm. Sci, in press, **2020**.





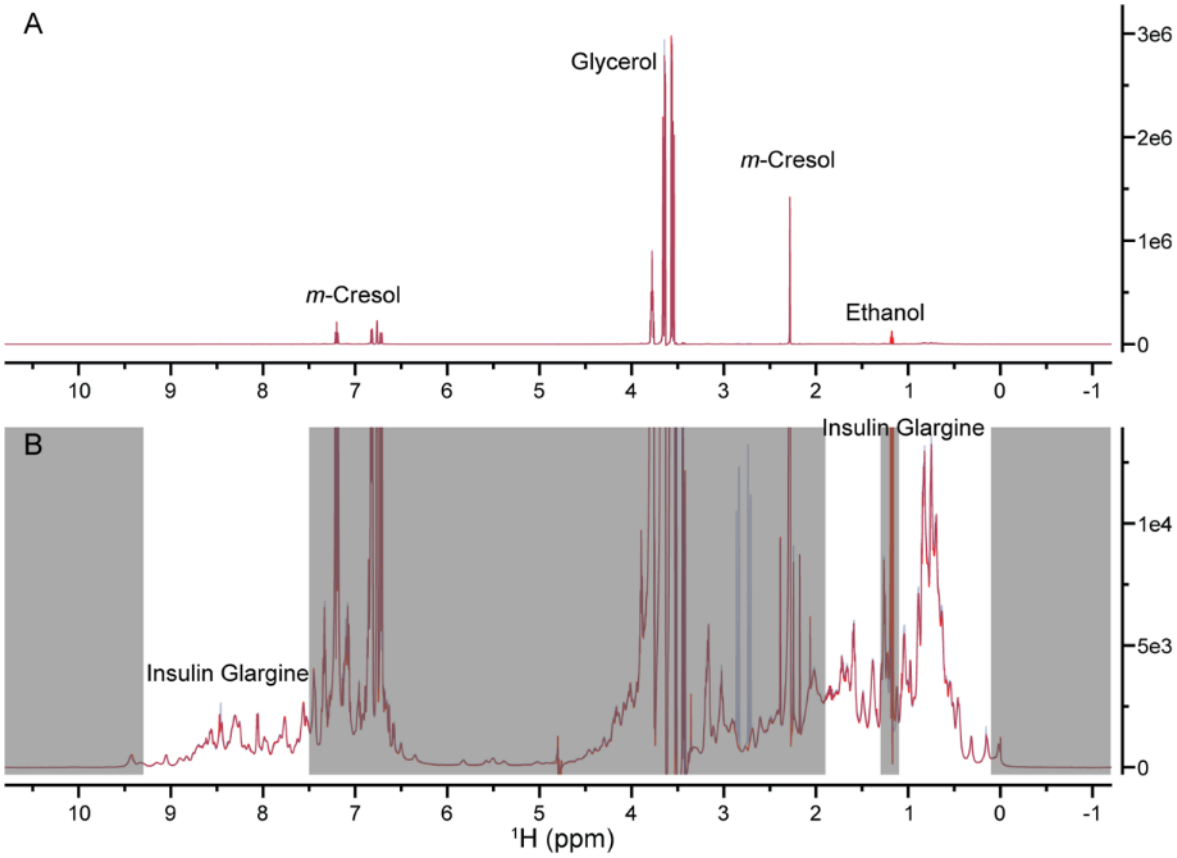
Humalog®

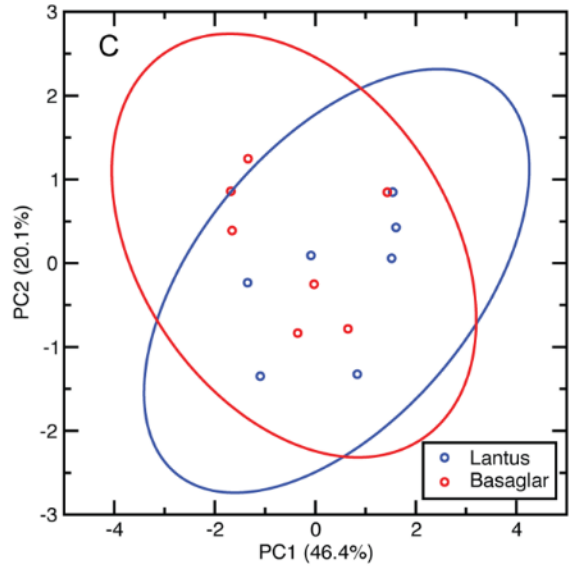
vs

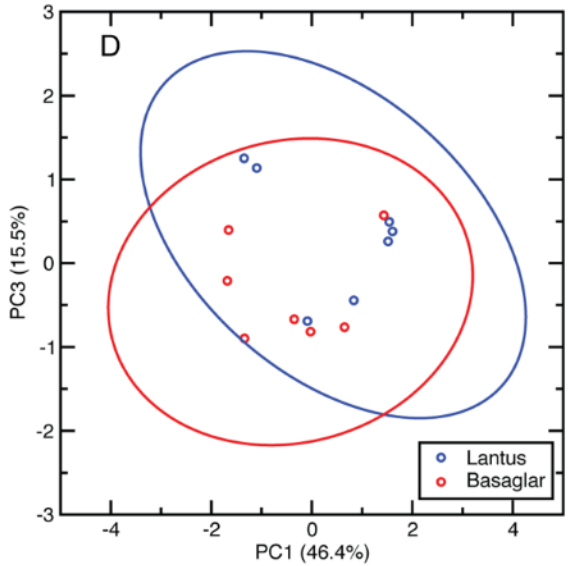
Admelog®

Dosage strength: 100 U/mL
(~ 0.6 mM)

Buffer: formulation + 5% D_2O



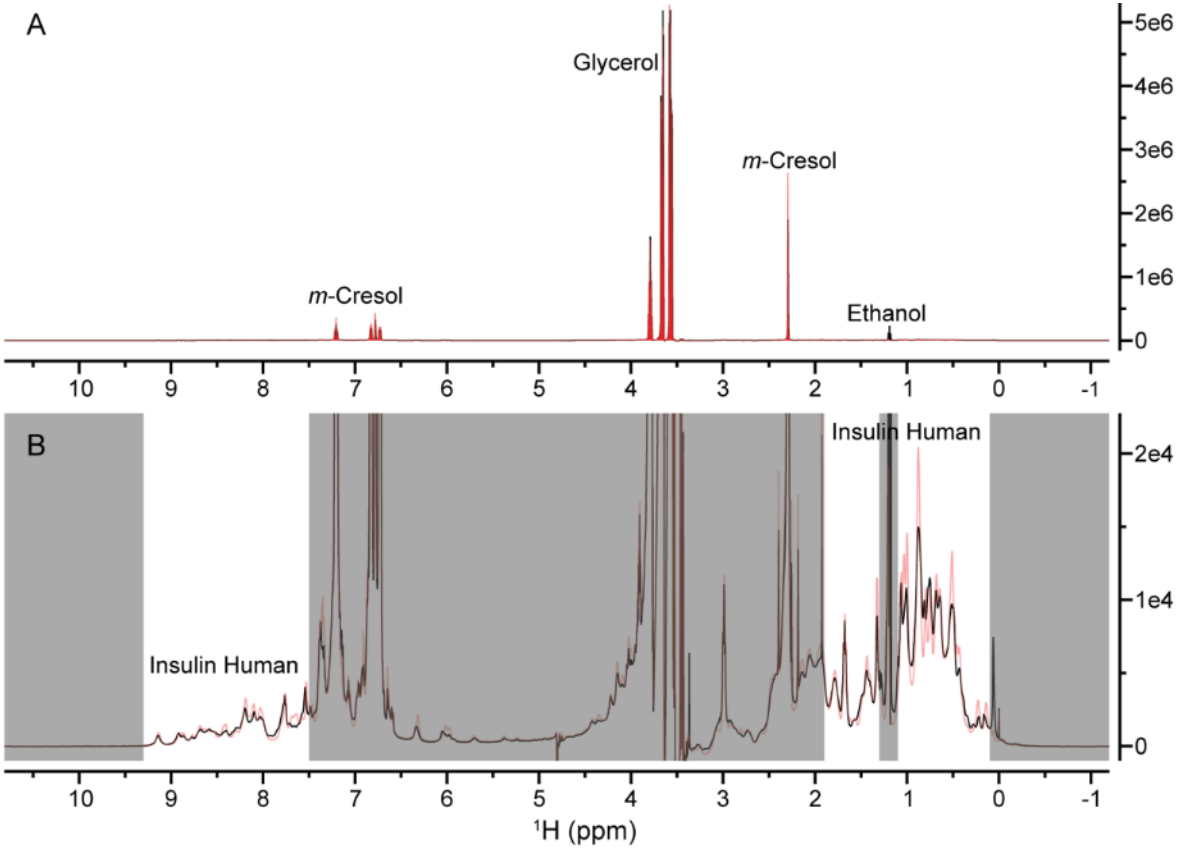




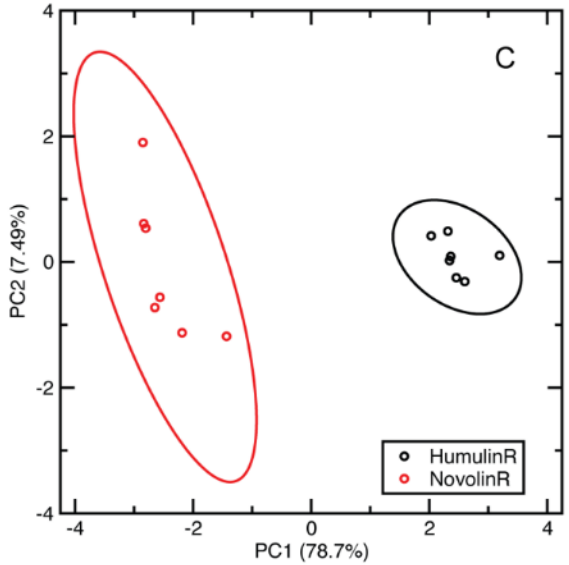
Lantus®
and
Basaglar®

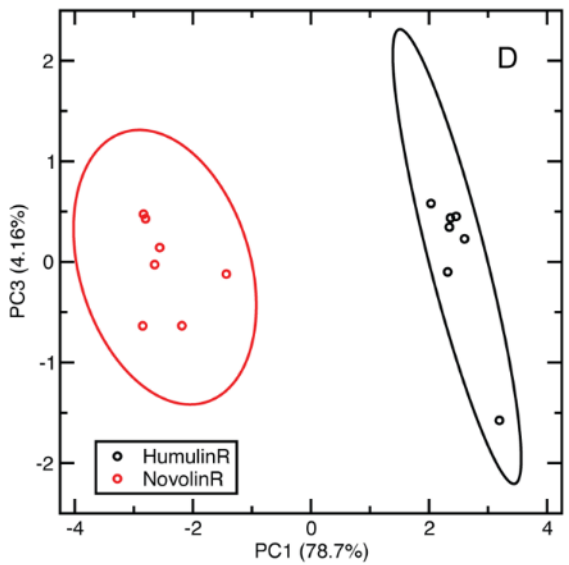
Dosage strength: 100 U/mL
(~ 0.6 mM)

Buffer: formulation + 5% D_2O



HumulinR® and NovolinR®

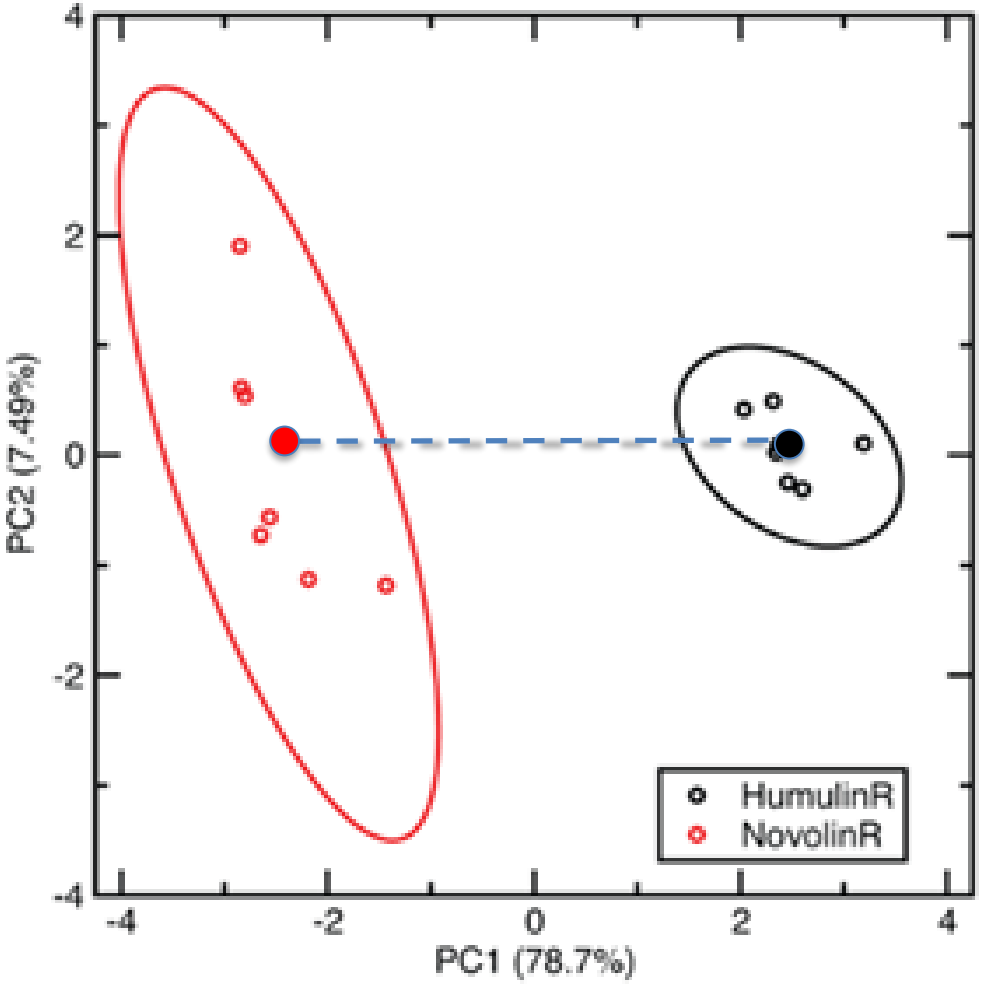




Dosage strength: 100 U/mL (~ 0.6 mM)

Buffer: formulation + 5% D_2O

Inter-brand Similarity



Mean vector of the HumulinR®

$$\bar{Z}_H = \left(\sum_{i=1}^{\mathrm{m}} Ha_i\right)/\mathrm{m}$$

Mean vector of the NovolinR®

$$\bar{Z}_N = \left(\sum_{i=1}^{\mathrm{n}} Na_i\right)/\mathrm{n}$$

Covariance matrices

$$S = (mS_H + nS_N)/(m+n)$$

Mahalanobis distance (D_M)

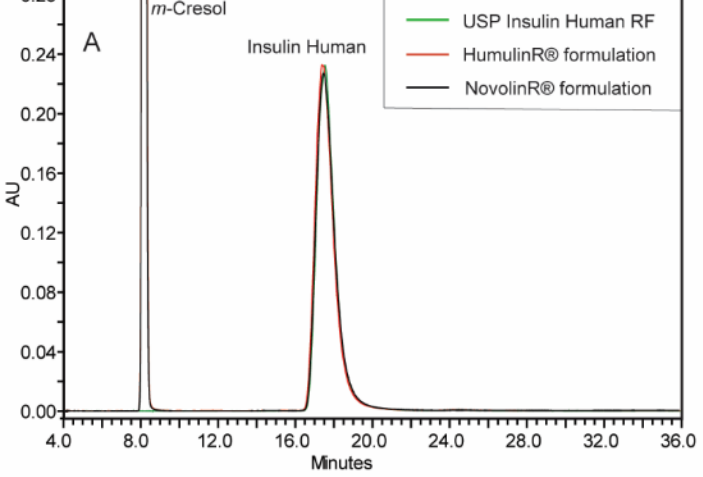
$$D_M = \mathrm{sqrt}[(\bar{Z}_H - \bar{Z}_N)S^{-1}(\bar{Z}_H - \bar{Z}_N)']$$

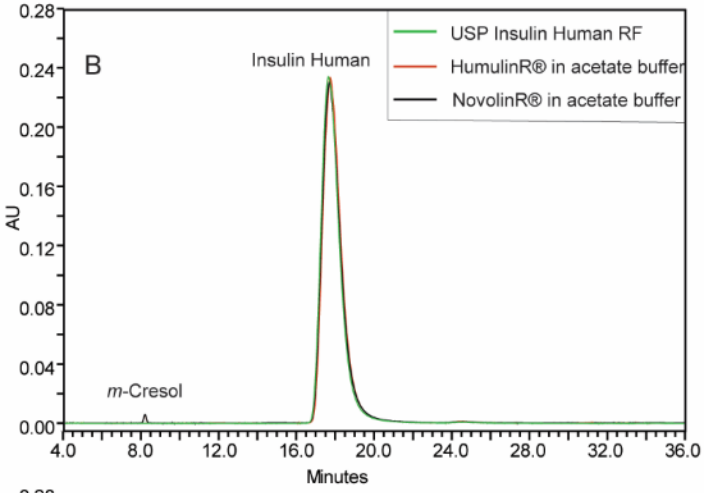
Chen K, Park J, Li F, Patil SM, Keire DA. *AAPS PharmSciTech*, 2018, 19(3):1011-1019.

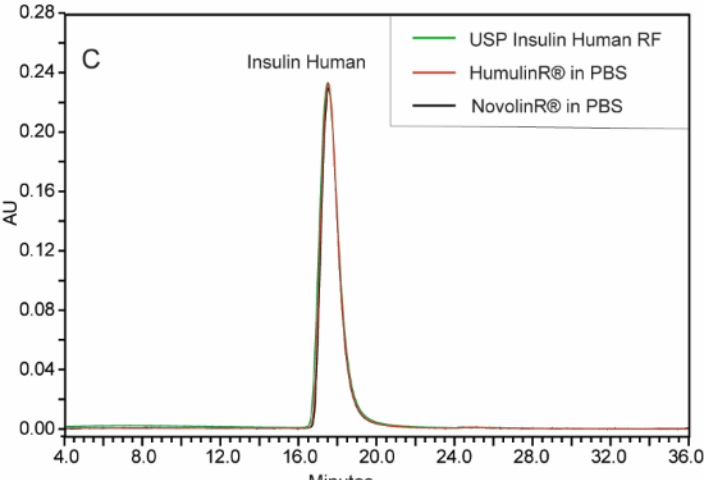
Achievable Similarity Metrics for Drug Products

Insulin Type	Drug Substance	Drug Product	Approval Type	Year approved	Inter-brand D_M
Rapid acting	Insulin Lispro	Humalog®	New Drug	1996	3.29
		Admelog®	Follow-on 505(b)(2)	2017	
Long acting	Insulin Glargine	Lantus®	New Drug	2000	1.58
		Basaglar®	Follow-on 505(b)(2)	2015	
Short acting	Insulin Human	HumulinR®	New Drug	1982	20.5
		NovolinR®	New Drug	1991	

Wang, D.; Park; Patil, S.; Smith, C.; Leaser, J.; Keire, D.; Chen, K., An NMR Based Similarity Metric for Higher Order Structure Quality Assessment among U.S. Marketed Insulin Therapeutics. J. Pharm. Sci, in press, **2020**.







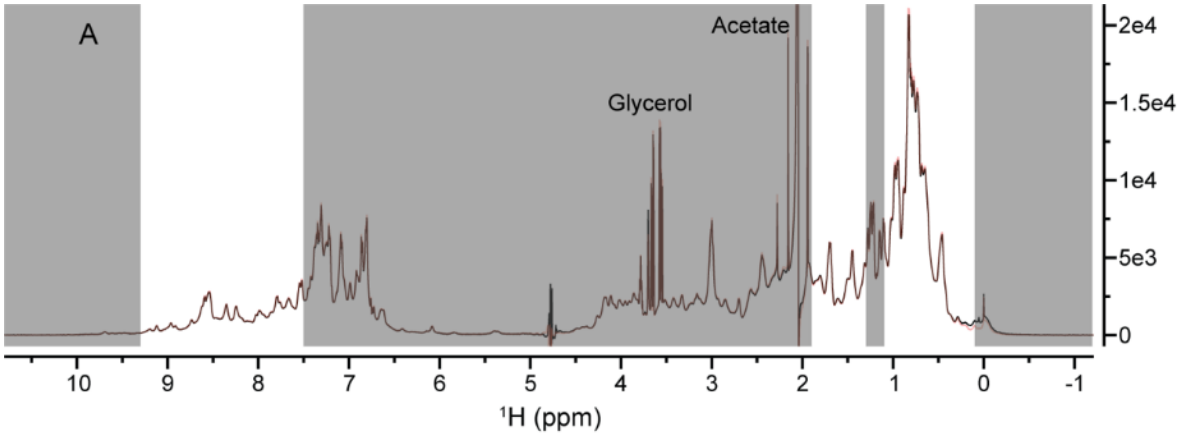
Approaches for Drug Substance HOS Similarity

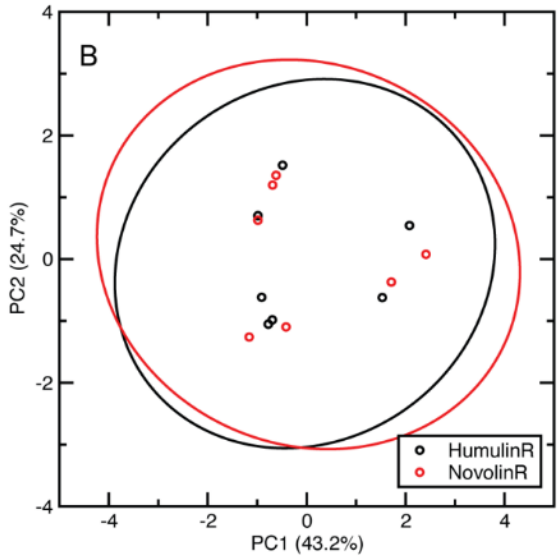
Assess whether HOS of DS would be convertible upon buffer exchange without loss of assay.

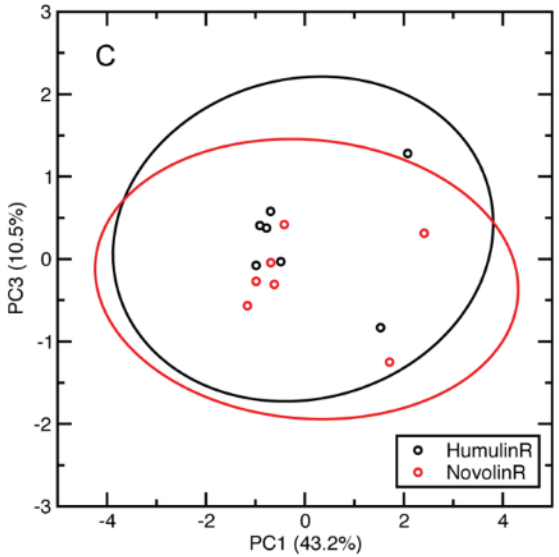
- Dialysis to 2 buffers
- Assure Mass-balance

Drug Product	Insulin Human assay (unit)	Buffer
HumulinR®	100.5	Sodium acetate (25 mM, pH 4.0)
NovolinR®	99.7	
HumulinR®	99.9	0.5x PBS (pH 7.4)
NovolinR®	98.6	

Insulin Human at pH 4

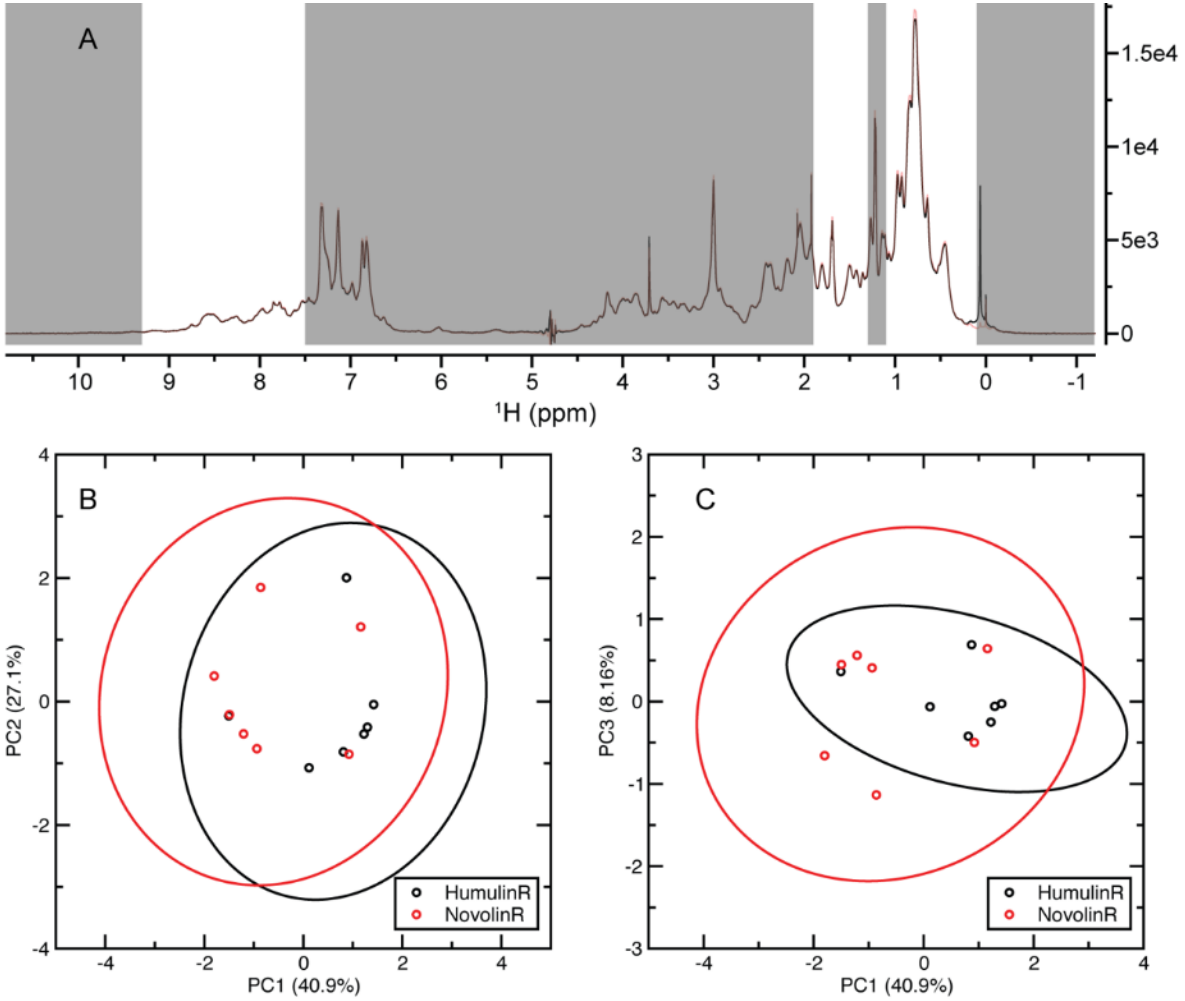






Drug Product	Buffer	Inter-brand D_M
HumulinR®	Sodium acetate (25 mM, pH 4.0)	0.818
NovolinR®		

Insulin Human at pH 7



Drug Product	Buffer	Inter-brand D_M
HumulinR®	0.5x PBS (pH 7.4)	1.19
NovolinR®		

Dm in Metabolomics

Table 1
Summary of Mahalanobis distances for cluster separations and Hotellings T^2 and F-test statistics for various datasets and pretreatment conditions.

	Mahalanobis distance	Two-sample T^2 statistic	F-value	Critical F-value	Significant?
No scaling					
Total separation	7.65	582.21	283.64	3.24	Yes
Partial separation #1	0.93	6.97	3.37	3.32	Yes
Partial separation #2	1.38	15.57	7.53	3.32	Yes
No separation	0.21	0.50	0.24	3.21	No

Quantification and statistical significance analysis of group separation in NMR-based metabonomics studies

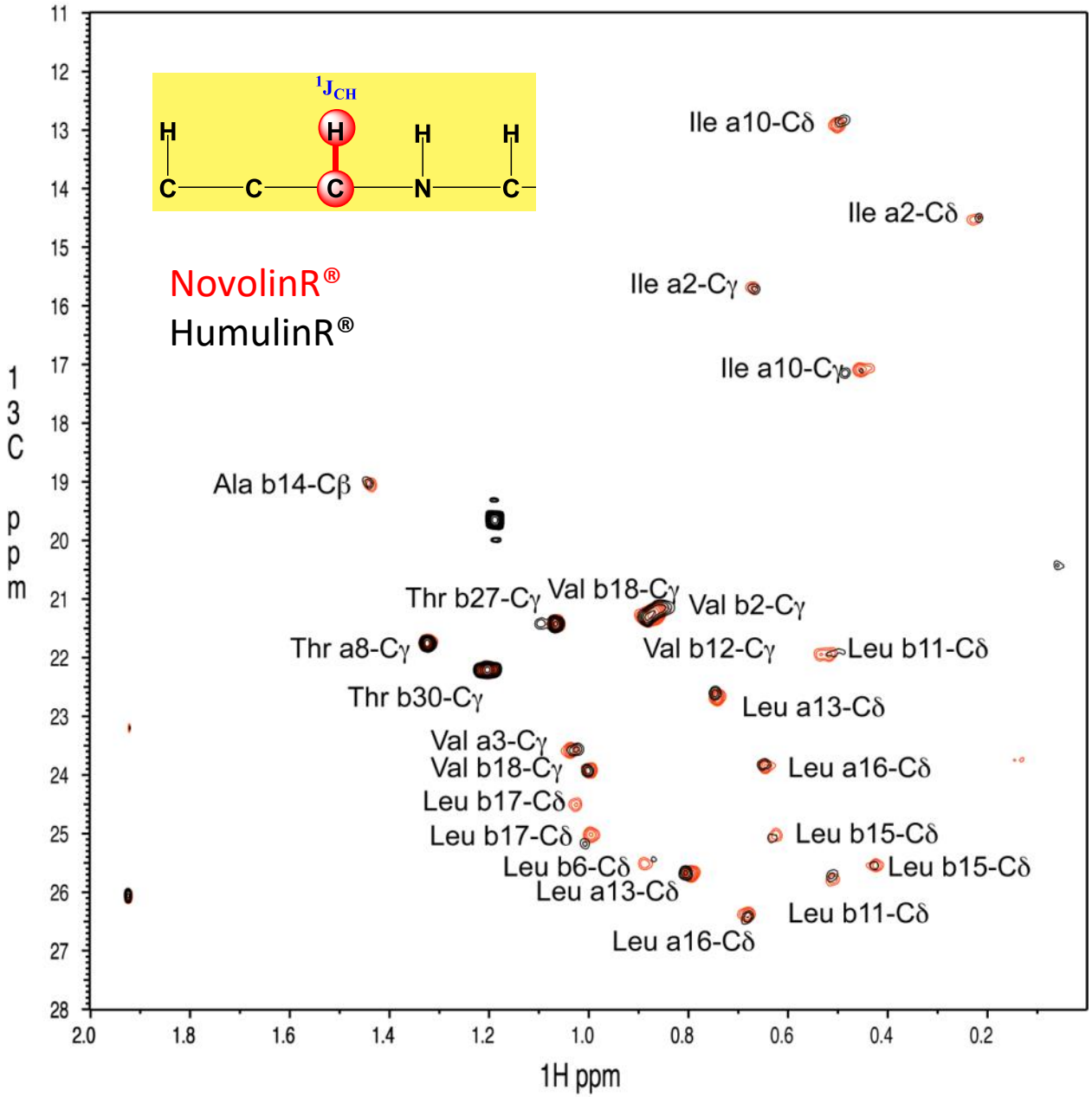
Aaron M. Goodpaster, Michael A. Kennedy *

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA

Further Understanding

- Similarity metrics like 8 ppb and Dm of 3.3 were achievable.
- For large difference in NMR spectra of Insulin Human in 2 drug products, which HOS quality is different? Possibly exchange kinetics.
- Can the NMR-PCA approach be applied to large protein or weakly concentrated peptide drugs? Yes.

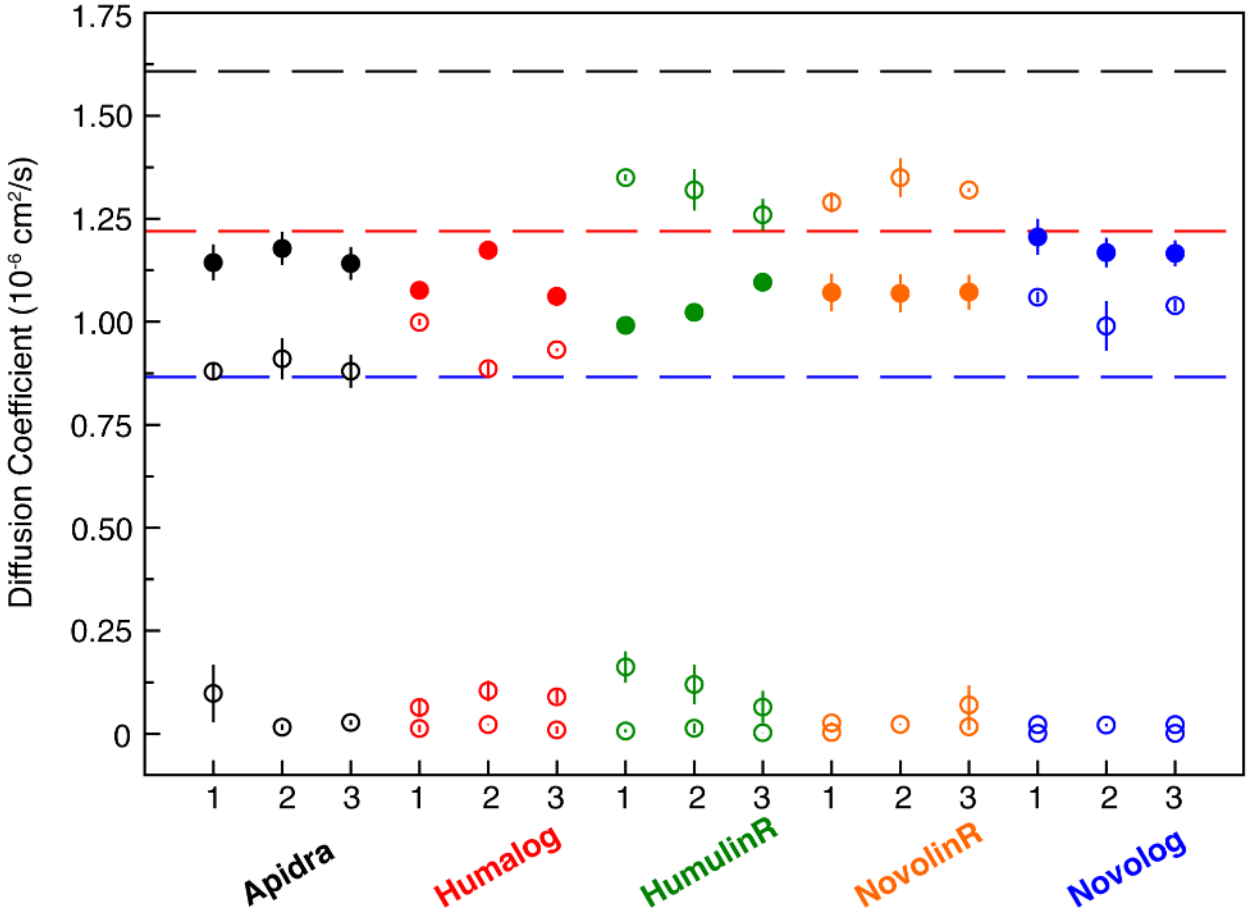
2D ^{1}H-^{13}C NMR



No major difference in 2nd structure.

Unpublished.

DOSY-NMR and DLS



monomer

dimer

hexamer

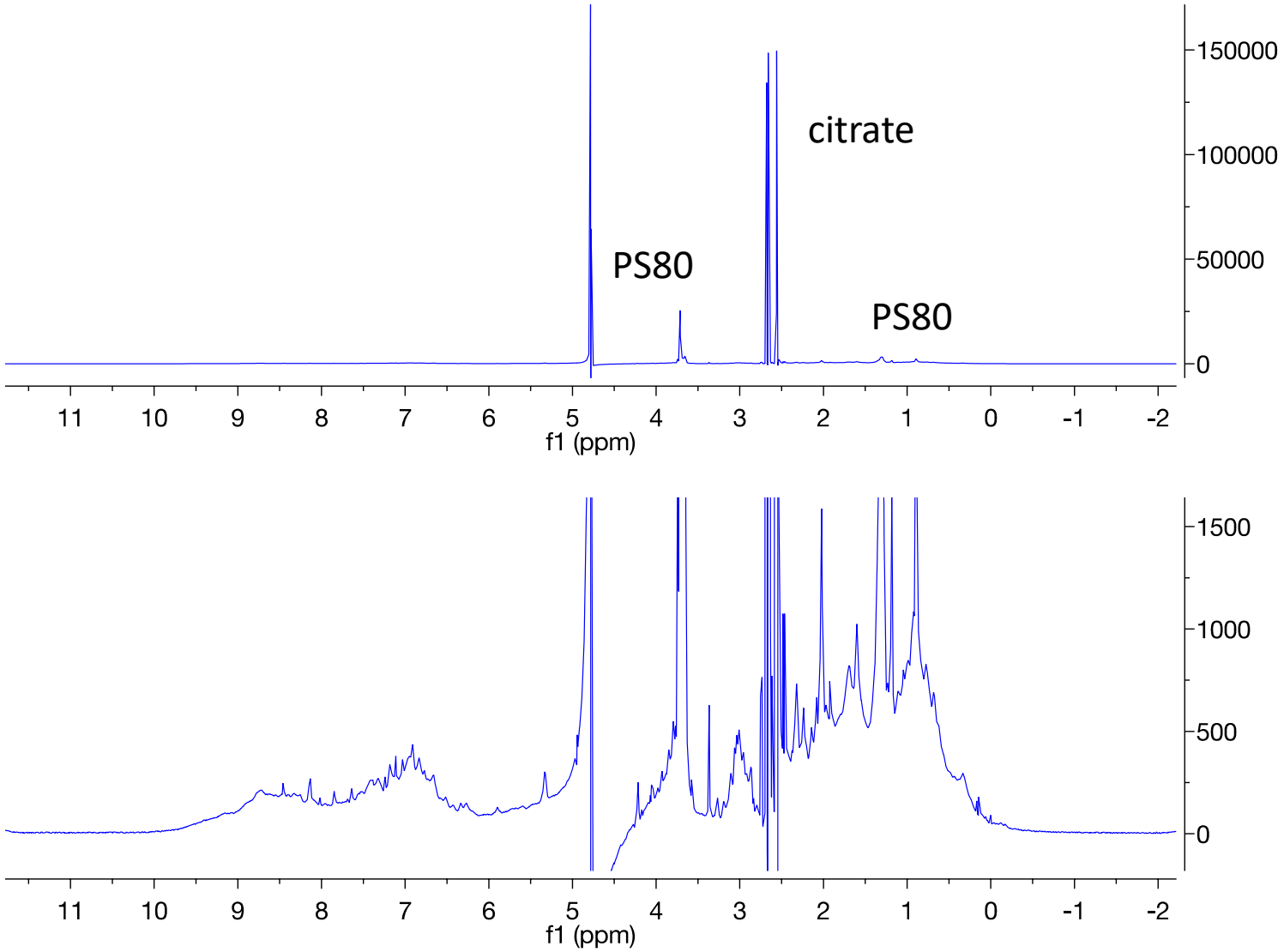
No major difference in oligomerization or aggregation states.

DOSY: filled circles

DLS: open circles (regularization fitting)

Patil, Keire and Chen 2017 AAPS-J

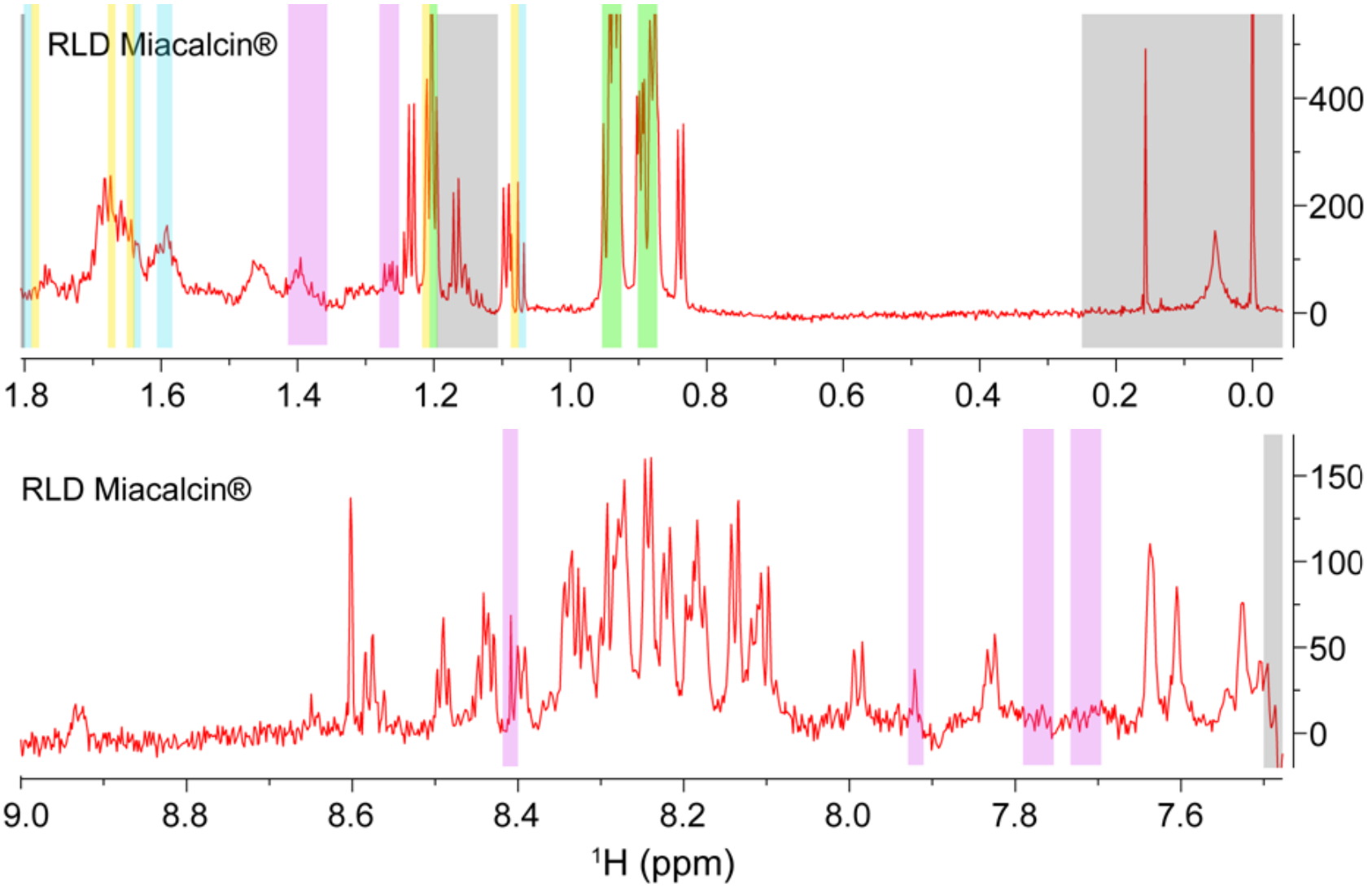
Rituximab Drug Product



Rituximab: 150 kDa, 10 mg/mL, ~ 0.067 mM

Unpublished

Calcitonin-salmon Drug Product



Calcitonin-salmon: 3.4 kDa, 200 IU/mL, ~ 9.7 μM

Unpublished

CONCLUSIONS

a. Unsupervised chemometric analysis on 1D NMR yielded robust similarity metrics: Mahalanobis distance (D_M). Values of similarity metrics for DP and DS were 3.3 and 1.2, respectively. These metrics were demonstrated on approved insulins, therefore, realistic and achievable.

b. NMR-PCA approach is generally applicable for other spectroscopy and complex or protein drugs.

c. DS comparison can be performed with HOS reversibility.

d. Detailed HOS change mechanism can be studied using 2D NMR and orthogonal methods.

Acknowledgement

FDA: Deyun Wang, Sharadrao Patil, Cameron Smith, John Leazer Jr and David Keire.

University of Maryland: Junyong Park

Discussions: Muthukumar Ramaswamy, Jeff Jiang, and Maria Gutierrez Lugo

Management: Jason Rodriguez, David Keire and Sau Lee