A Path Forward: Establishing New NMR Analytical Protocols to Assure the Quality of Biologics

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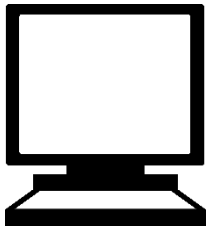
WCBP January 28th, 2020

This presentation reflects the views of the author and should not be constructed to represent FDA's views or polices.

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.

Biosimilarity 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

CLASSICAL HOS TESTING

Low to medium resolution biophysical techniques:

- Fourier Transform Infrared
- Circular Dichroism
- Intrinsic Fluorescence
- Differential Scanning Calorimetry

STATE OF THE ART

High resolution biophysical techniques:

- High Field NMR Spectroscopy
  - 500 MHz or higher
  - Multiple potential techniques (2D-H,X-HSQC, 1D Profile)
- High Resolution Mass spectrometry
  - Hydrogen Deuterium Exchange-MS
  - Ion-Mobility MS
  - Multiple Attribute Monitoring (MS Workflow)

WHY CHANGE?

- The existing tests are fine and have been used for drugs that are approved and on the market.
- Nobody understands this mountain of data.
- Maybe a new technology/measurement is better but that does not mean it will improve the quality of my drug.
- Costs to much.

THE GUIDANCE IS THERE FOR A REASON

- Biologic drugs are multi-attribute drugs and many features can impact function (e.g., a folded structure, a glycosylation, a deamidation or an oxidation).
- If you are not measuring an attribute then you may not know about a change in that property and will not be able to understand the impact of a change.

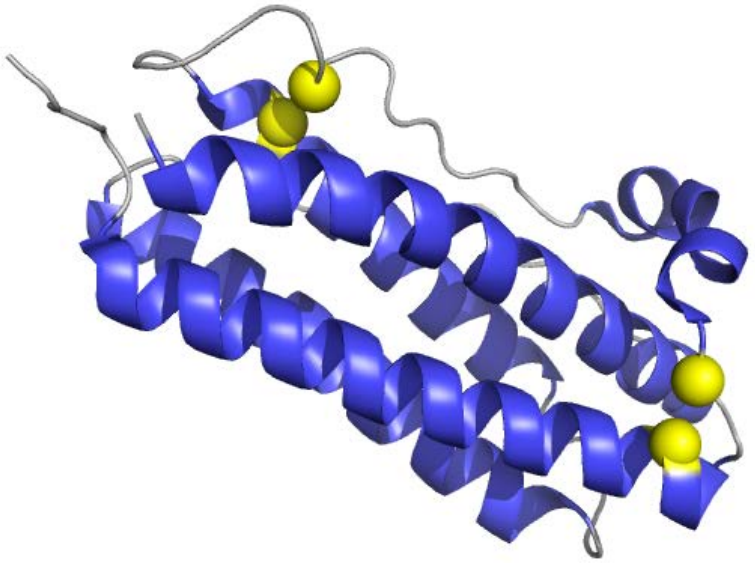
What you don't measure/control may lead to unintended consequences.
Loss of potency or efficacy
Pathological action

Higher Resolution/Higher Sensitivity Data → Improved Product Risk Assurance (Increased Product Knowledge).
Long Term Thinking: No Data Wasted.

Higher Order Structure Matters

The folded structure of filgrastim is necessary for normal activity.

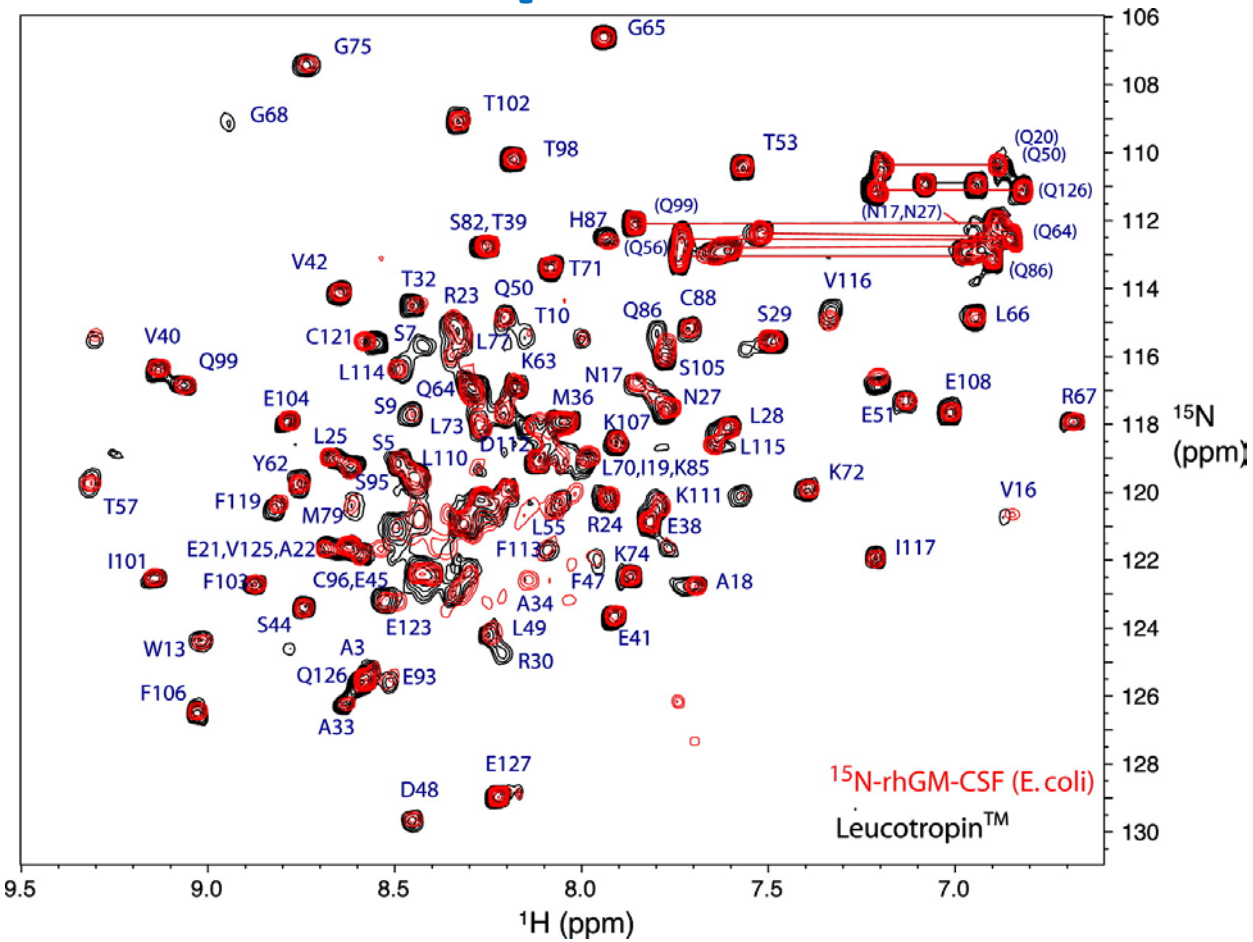
Mis-folded structures could lead to no activity or pathogenic function.



The non-glycosylated version of G-CSF is called filgrastim and is used therapeutically for cancer patients on chemotherapy to help maintain white blood cell counts and prevent infections.

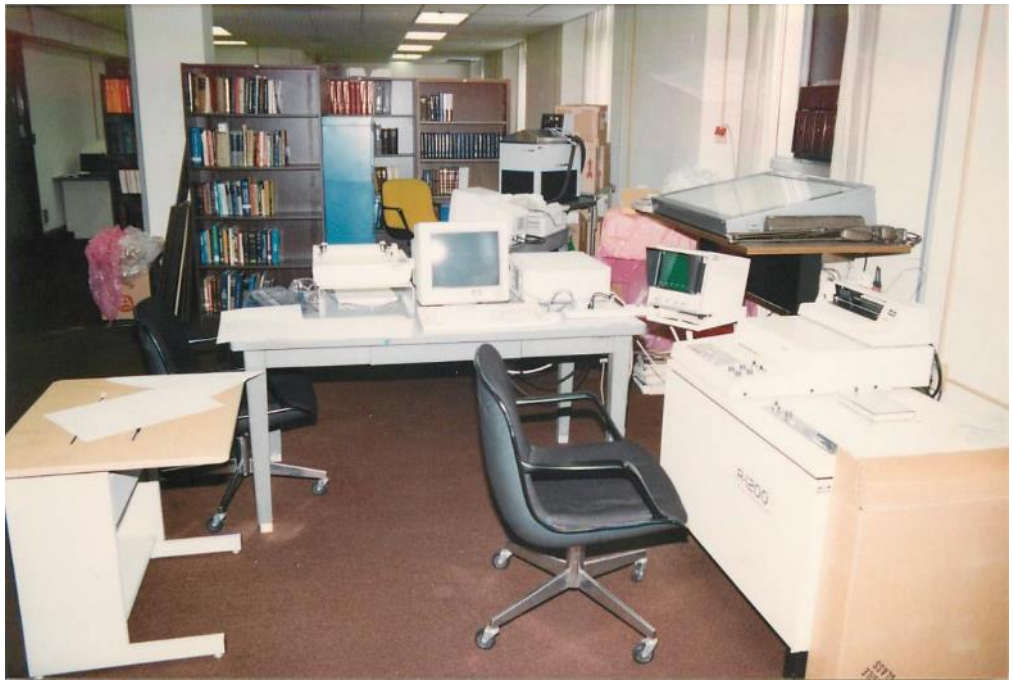
NMR for HOS Example

First demonstration of native isotopic abundance NMR on a protein therapeutic



Aubin Y, Gingras G, Sauvé S. Assessment of the three-dimensional structure of recombinant protein therapeutics by NMR fingerprinting: demonstration on recombinant human granulocyte macrophage-colony stimulation factor. Anal Chem. 2008;80(7):2623–2627. doi:10.1021/ac7026222.

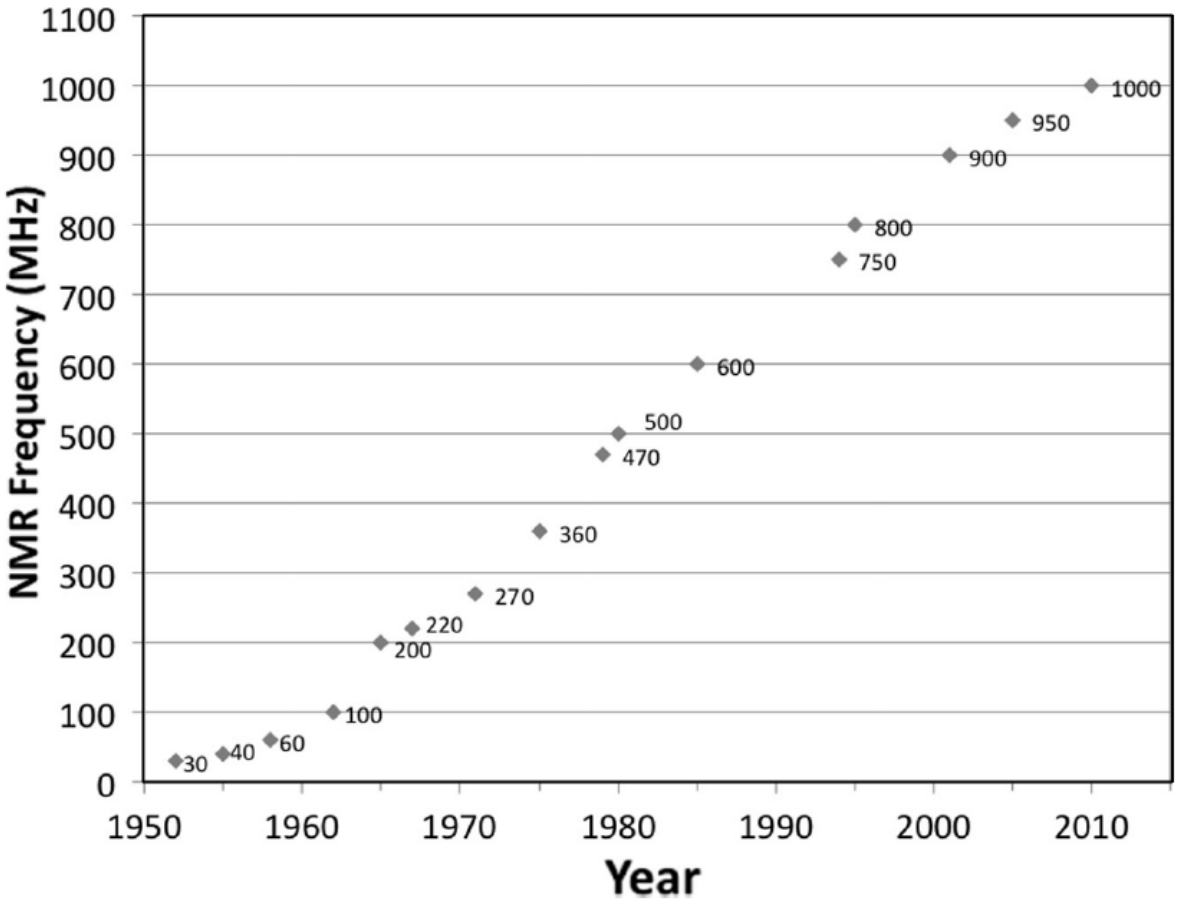
FDA St Louis Lab 2008



Hitachi R1200 60 MHz Instrument

NMR has evolved

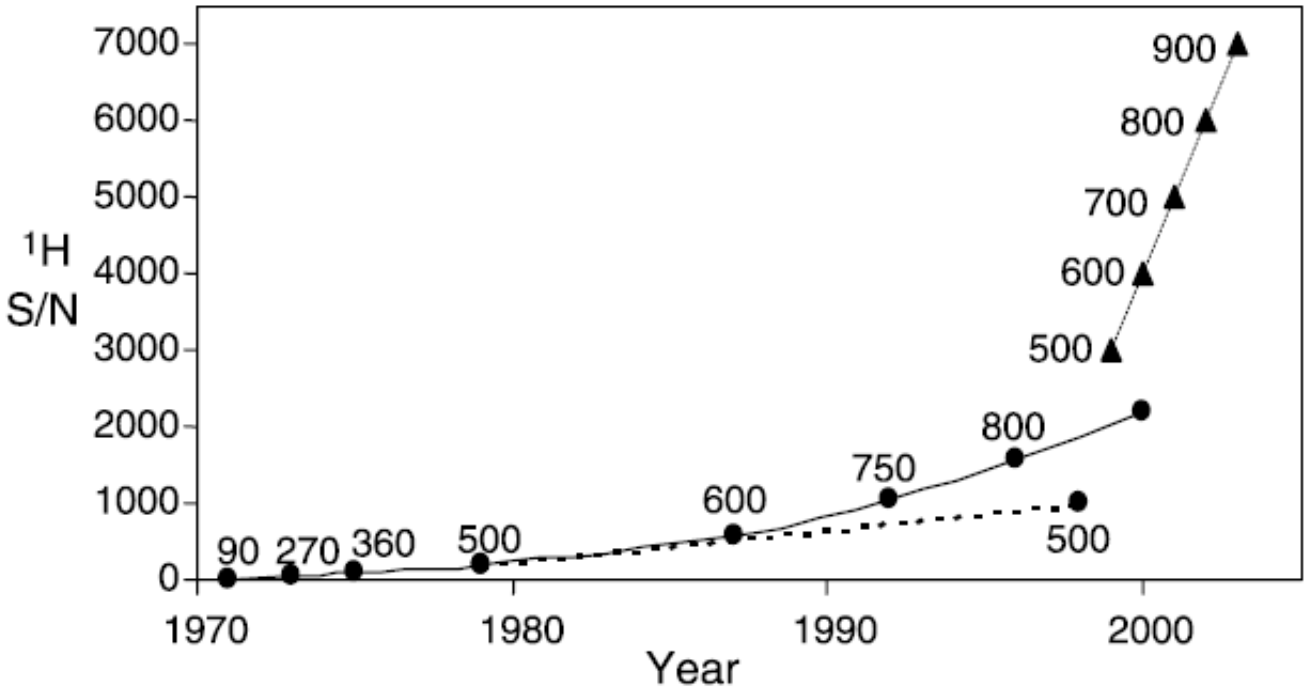
~20 MHz/y



A similar curve could be drawn for resolution in MS instruments

Wishart, Trends in Anal Chem., 48, 96-111, 2013

NMR Sensitivity (1H-EB S/N)



Kovacs *et al.,* PiNMRS, 46, 131-151, 2005

Inter-laboratory Comparability Study: FDA, NIST, Health Canada and MPA-Sweden

FDA

LÄKEMEDELSVERKET
MEDICAL PRODUCTS AGENCY

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

Health Canada Santé Canada

(Filgrastim; Neupogen®)

www.fda.gov

Round robin study on the similarity of NMR spectral 'fingerprints' obtained using standardized 2D ^{1}H-^{15}N HSQC experiments

4 Sites in North America and Europe

FDA; Health-Canada; MPA-Sweden; NIST

4 Fields – Six spectrometers

500, 600, 700 and 900 MHz

Different Instrument vintages

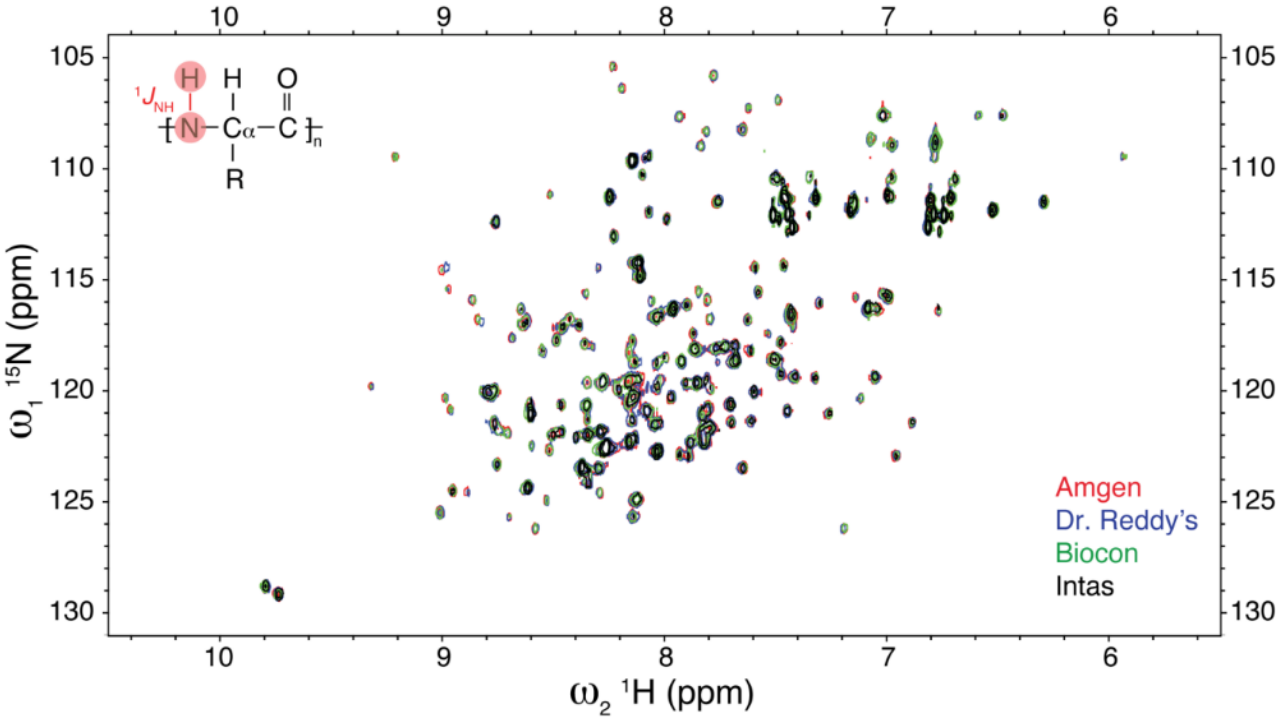
2 Vendors

Bruker Biospin, Varian/Agilent

Overlay of the 2D ^{1}H-^{15}N HSQC spectra from 4 filgrastim products at natural abundance

Amgen's Neupogen (US)
Biocon's Nufil (Foreign)
Dr. Reddy's Grafeel (Foreign)
Intas Neukine (Foreign)

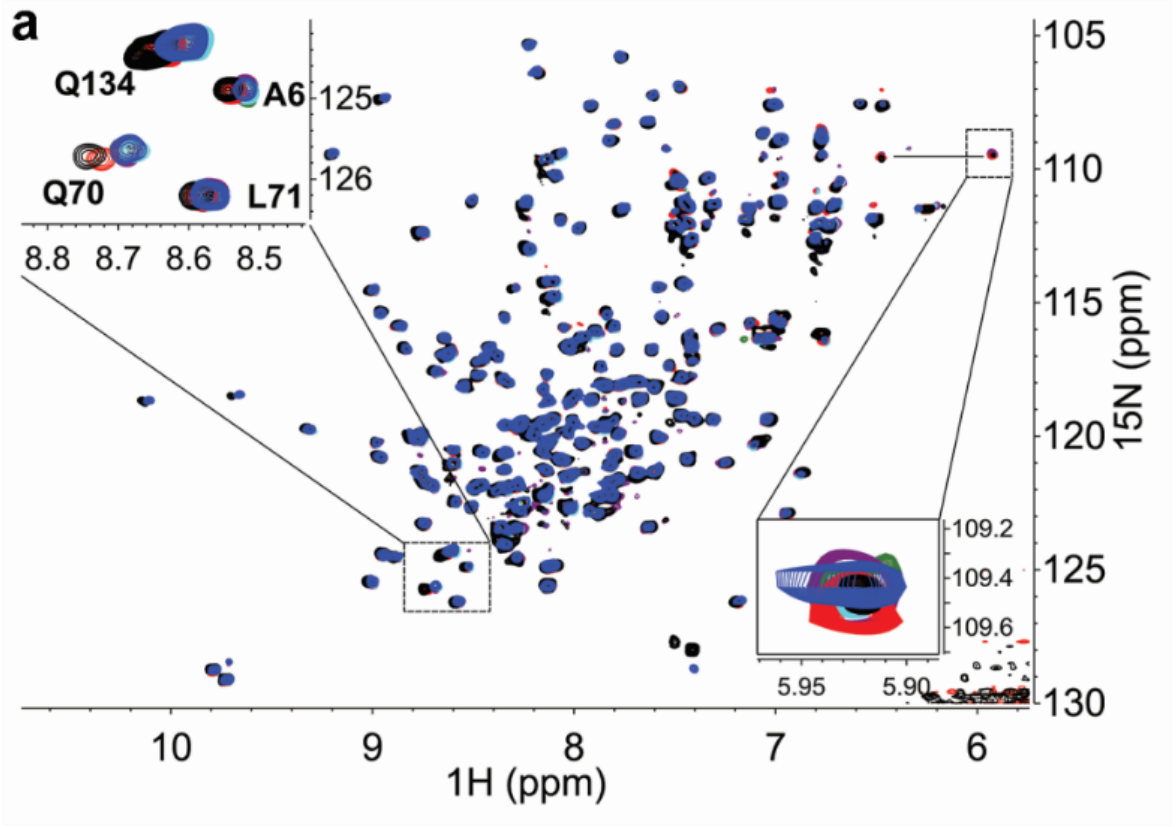
^{15}N-Labeled version from Health Canada



Data from cryogenic probe on NIST900

Overlay of the 2D ^{1}H-^{15}N HSQC spectra of Neupogen® at varied magnetic fields

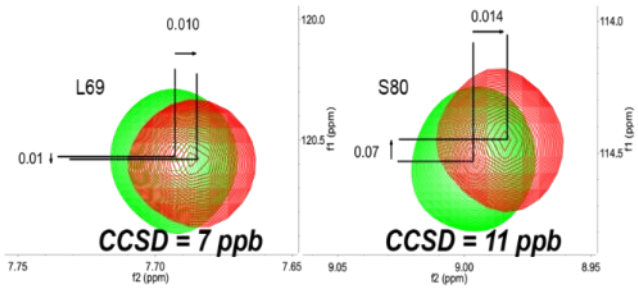
46 hrs on cryo-900
89 hrs on cryo-500
S/N > 10



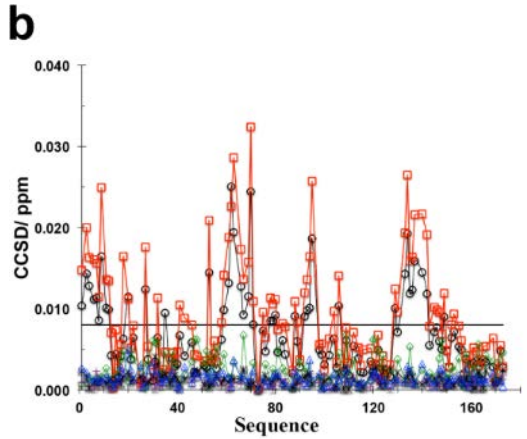
www.fda.gov

Ghasriani H, Hodgson DJ, Brinson RG, et al. Precision and robustness of 2D-NMR for structure assessment of filgrastim biosimilars. Nat Biotechnol. 2016;34(2):139–141. doi:10.1038/nbt.3474

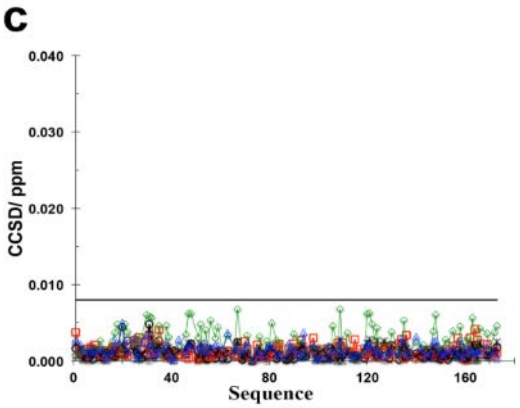
Quantifying Chemical Shift Difference



CCSD=
"combined chemical shift difference"
$\sqrt{[0.5*(\delta_H^2+(\alpha * \delta_N)^2)]}$



8 ppb



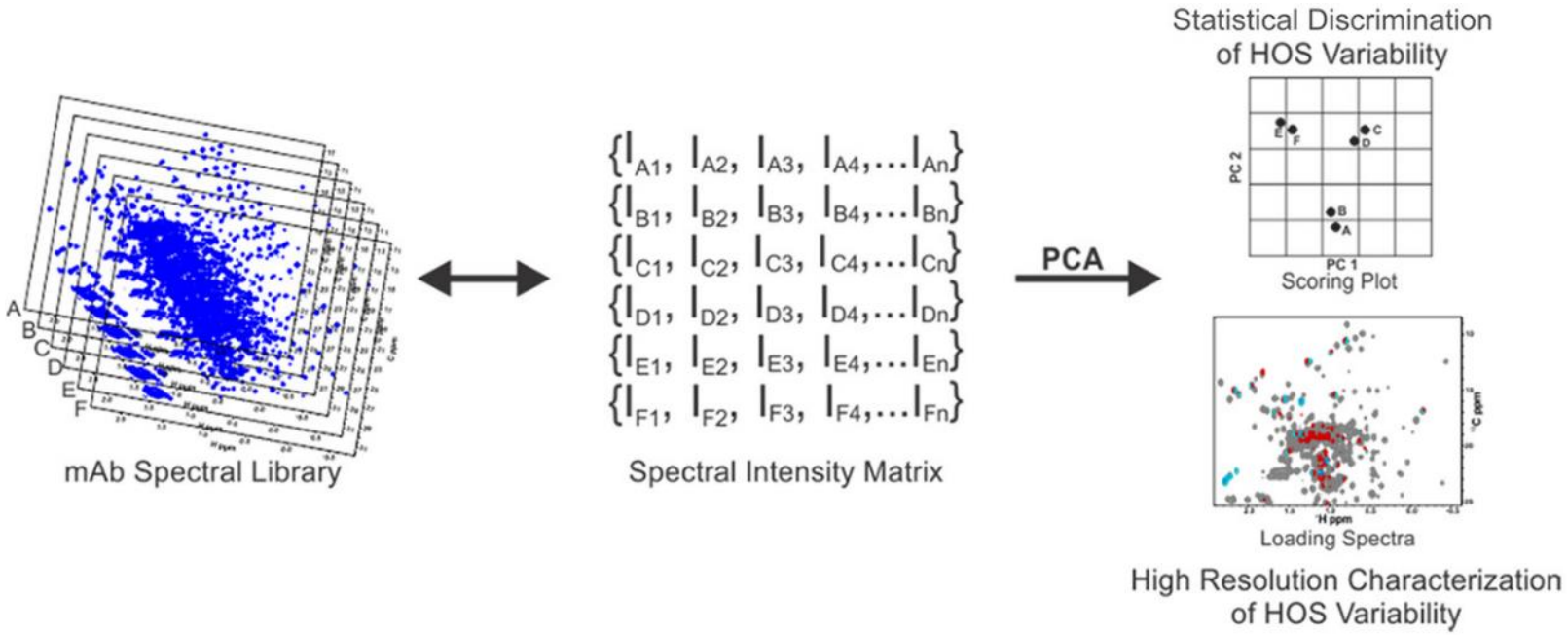
8 ppb

- Sensitive
- Need peak picking
- Ignore peak height/intensity

www.fda.gov

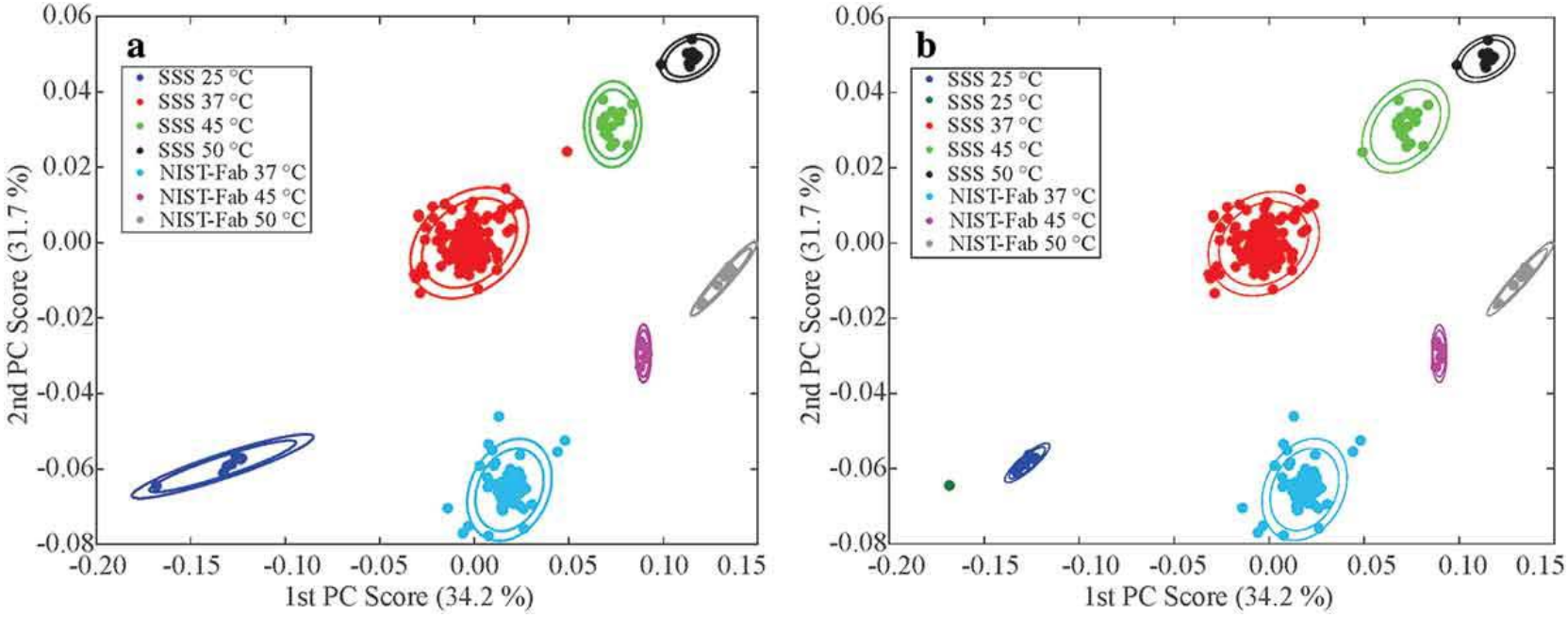
FDA 2018

Multi-Lab (26), Field (39) NISTmAb Study



Brinson RG, Marino JP, Delaglio F, et al. Enabling adoption of 2D-NMR for the higher order structure assessment of monoclonal antibody therapeutics. *MAbs*. 2019;11(1):94–105. doi:10.1080/19420862.2018.1544454

MULTI-LAB (26), FIELD (39) NISTMAB STUDY



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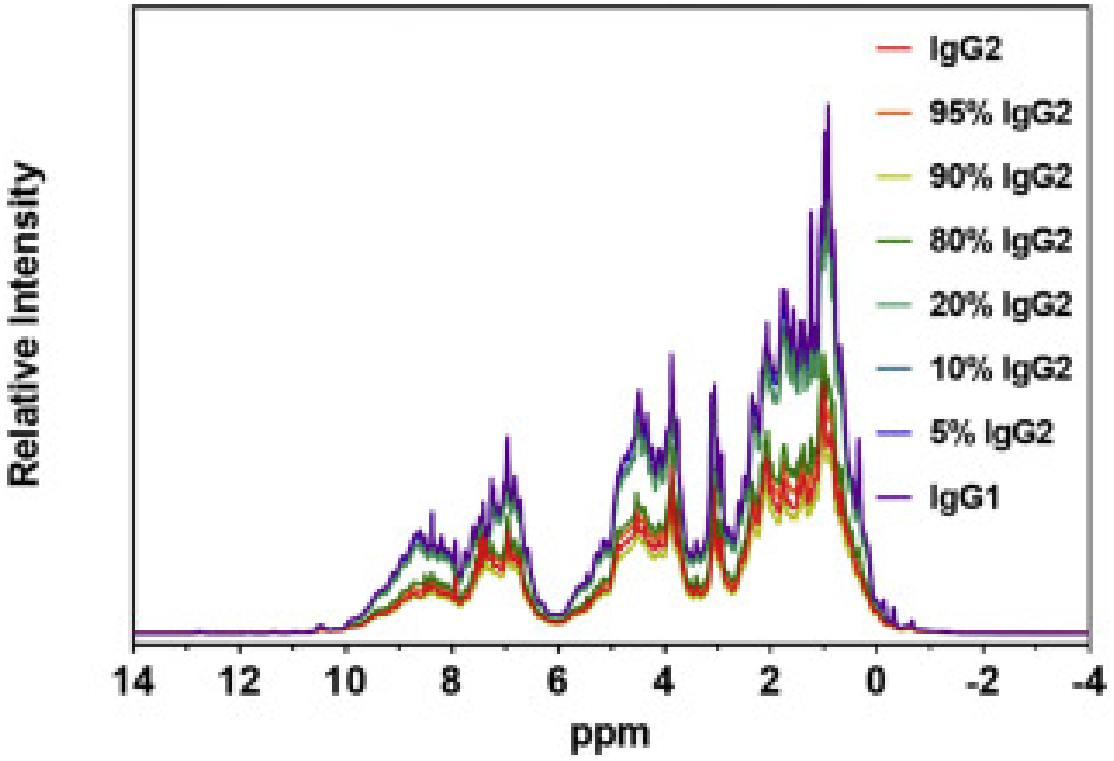
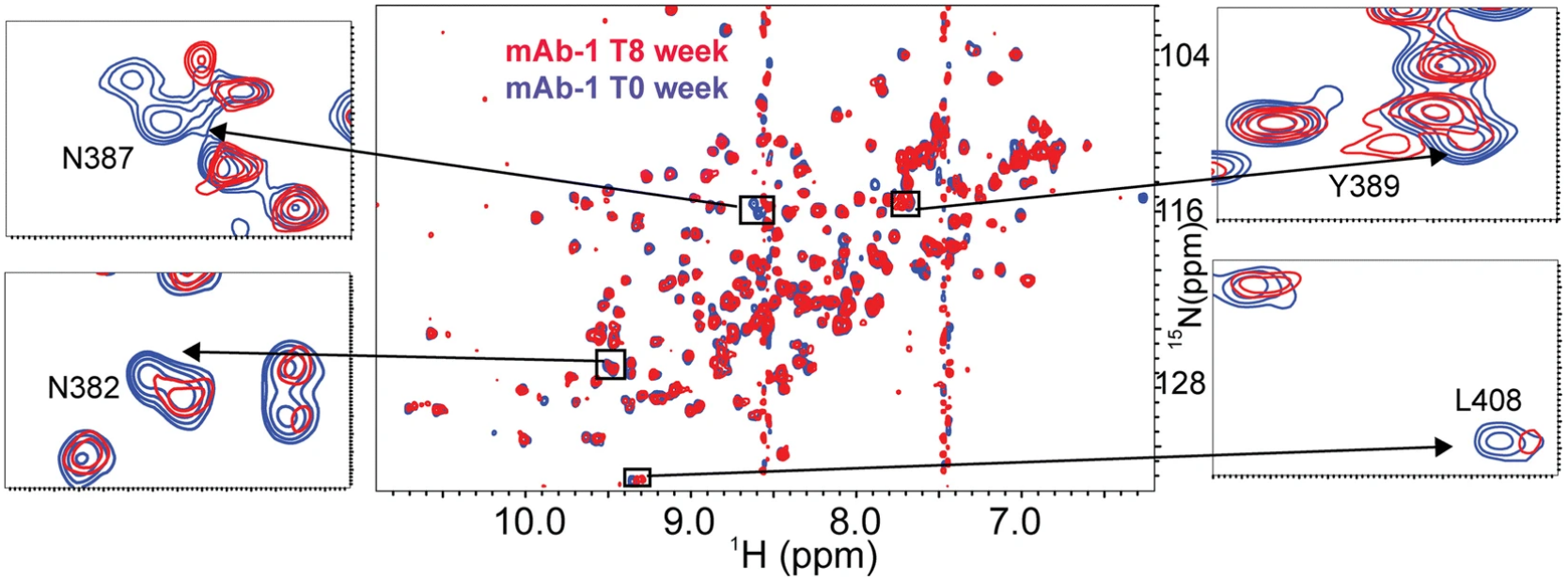
1D PROFILE (AMGEN)

Compared to “classical” techniques and was more discriminating



Wen J, Batabyal D, Knutson N, Lord H, Wikström M. A Comparison Between Emerging and Current Biophysical Methods for the Assessment of Higher-Order Structure of Biopharmaceuticals. J Pharm Sci. 2020;109(1):247–253. doi:10.1016/j.xphs.2019.10.026

HOS OF MABS AT PFIZER



Proposed an integrated approach to relate chemical modification to HOS. Potential connection of HOS modification to loss of potency.

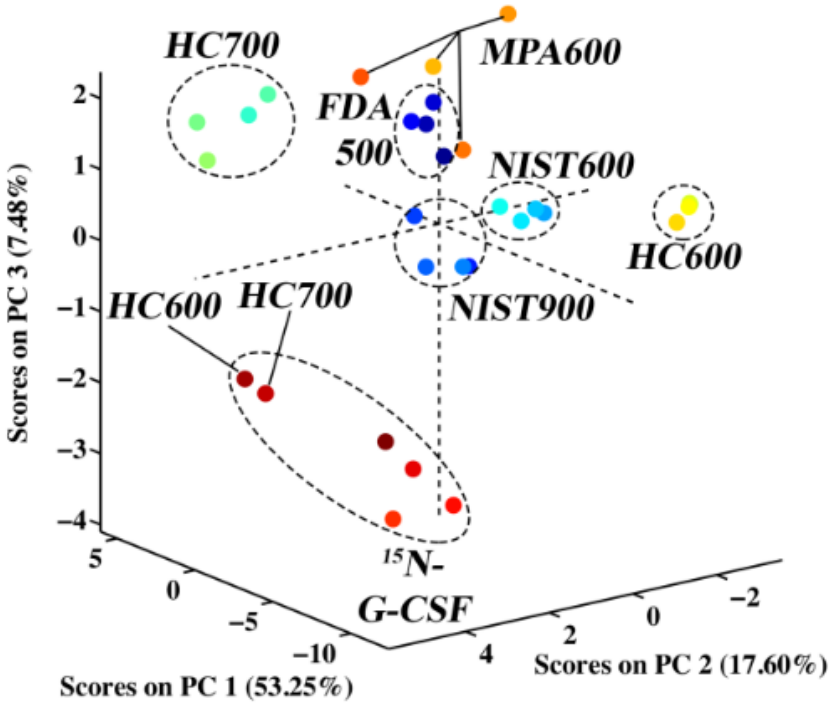
Majumder S, Saati A, Philip S, et al. Utility of High Resolution NMR Methods to Probe the Impact of Chemical Modifications on Higher Order Structure of Monoclonal Antibodies in Relation to Antigen Binding. Pharm Res. 2019;36(9):130. Published 2019 Jul 1. doi:10.1007/s11095-019-2652-1

So I have a boat full of data, now what do I do?

Smarter Analysis

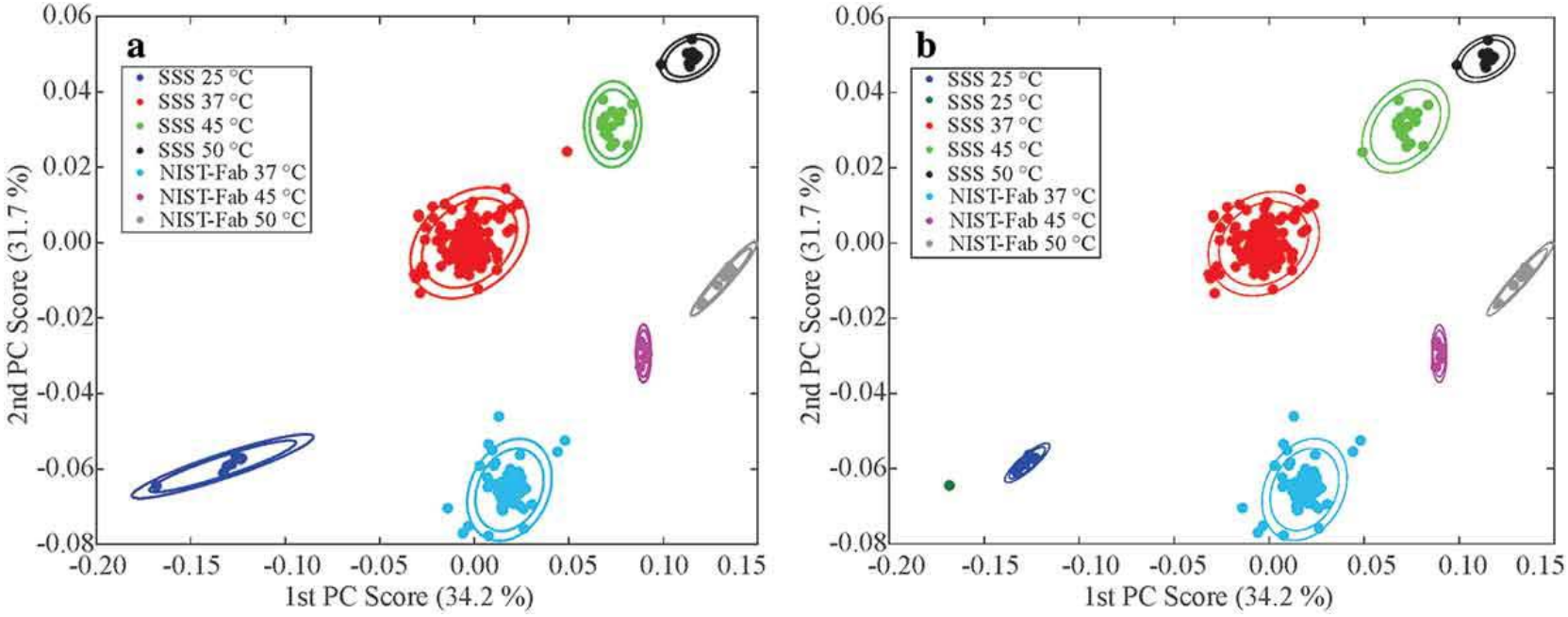
Principal Component Analysis (PCA)

- These approaches can use all the data rather than specific peaks.
- They can use a library of "good" drug spectra to detect outliers.
- They can potentially remove the expert from routine analyses.
- They are unbiased and do not have a bad day.



Ghasriani, Hodgson, et al., 2016 Nature Biotechnology

MULTI-LAB (26), FIELD (39) NISTMAB STUDY



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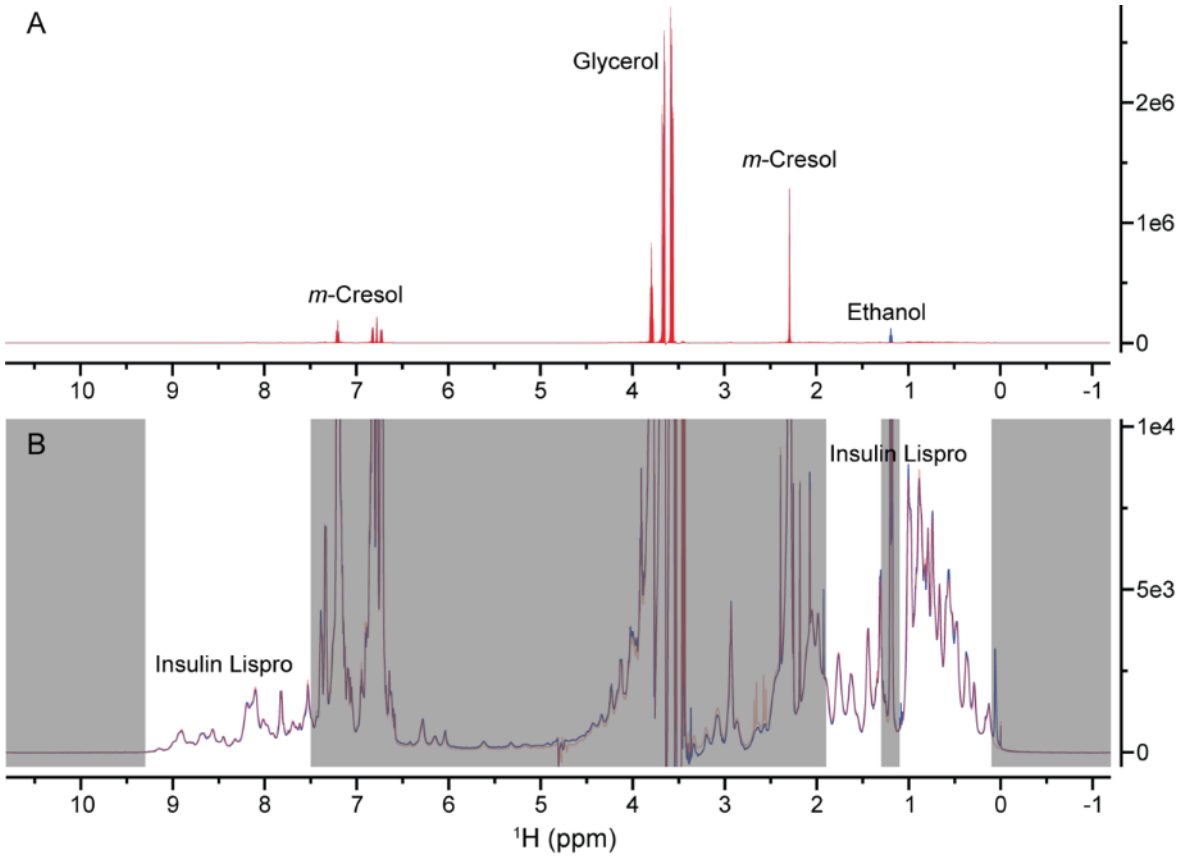
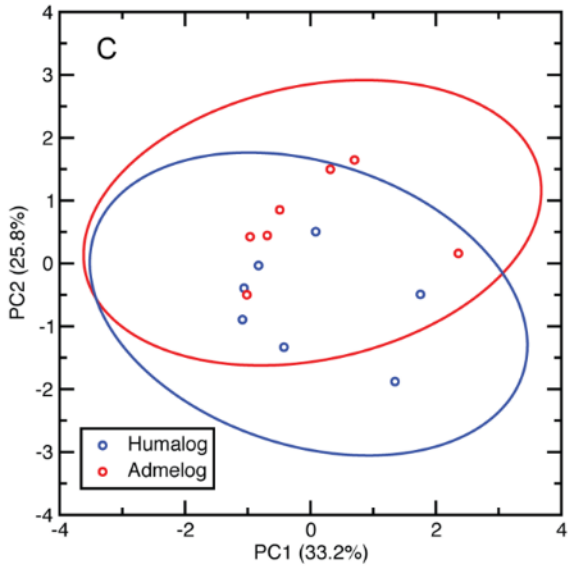
Metrics for Similarity

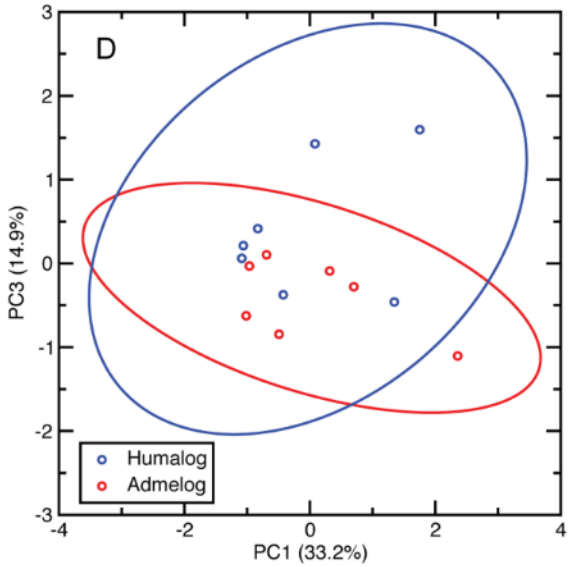
- Chen K., Park J., Li F., Patil S.M. and **Keire D.A.**, "Chemometric Methods to Quantify 1D and 2D NMR Spectral Differences among Similar Protein Therapeutics", *AAPS-PharmSciTech,* 19(3), 1011-1019 **(2018)**.
- Wang D., Park J., Patil S.R., Smith C.J., Leazer J.L., **Keire D.A.,** Chen K. "An NMR Based Similarity Metric for Higher Order Structure Quality Assessment among U.S. Marketed Insulin Therapeutics", *J. Pharm. Sci., Accepted for publication,* **January (2020)**.

Some of US Marketed Insulin DPs

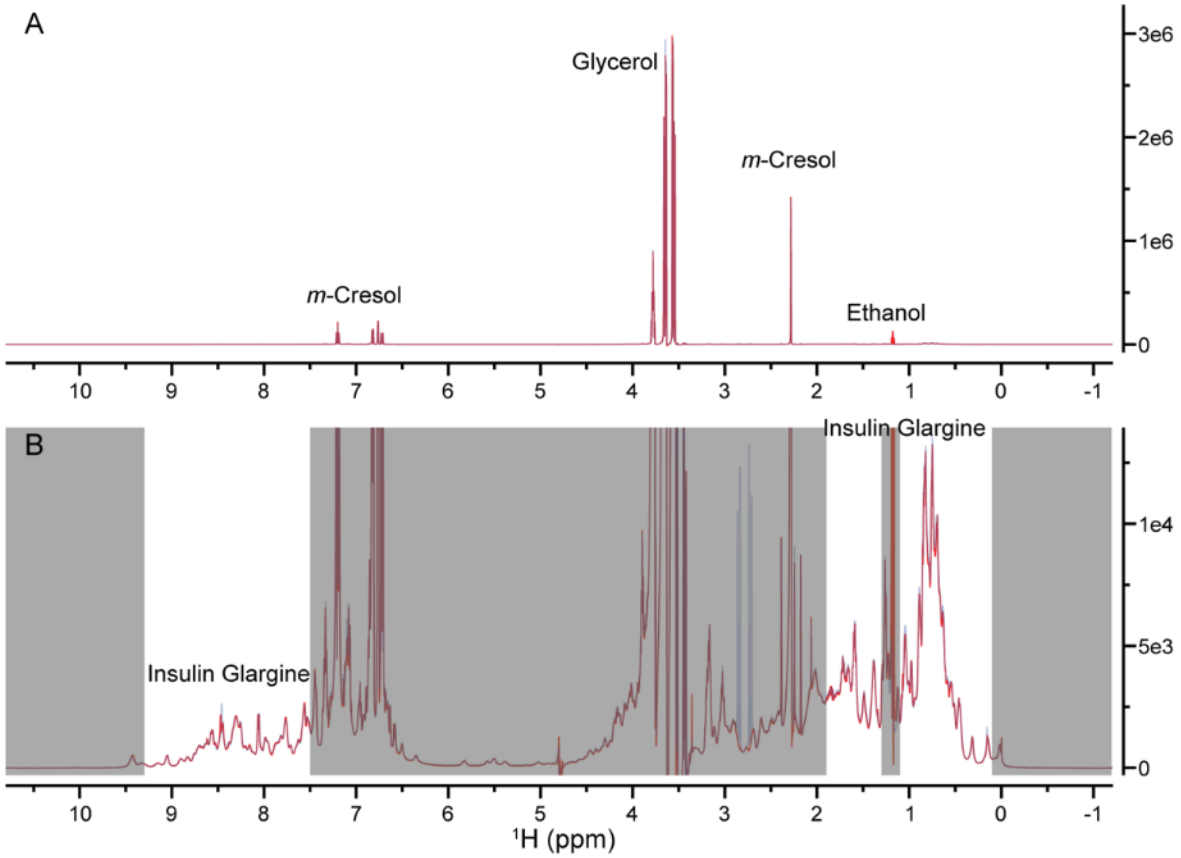
Insulin Type	Drug Substance	Drug Product	Approval Type	Year approved	Buffer
Rapid acting	Insulin Lispro	Humalog®	New Drug	1996	100 U/mL Intact formulation
		Admelog®	Follow-on 505(b)(2)	2017	
Long acting	Insulin Glargine	Lantus®	New Drug	2000	
		Basaglar®	Follow-on 505(b)(2)	2015	
Short acting	Insulin Human	HumulinR®	New Drug	1982	
		NovolinR®	New Drug	1991	

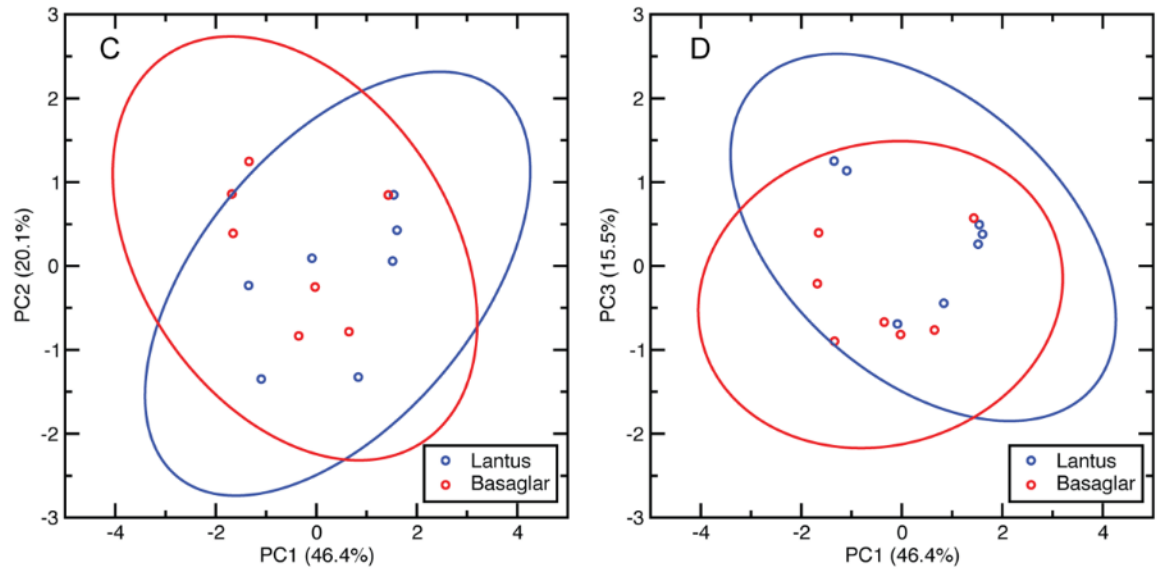




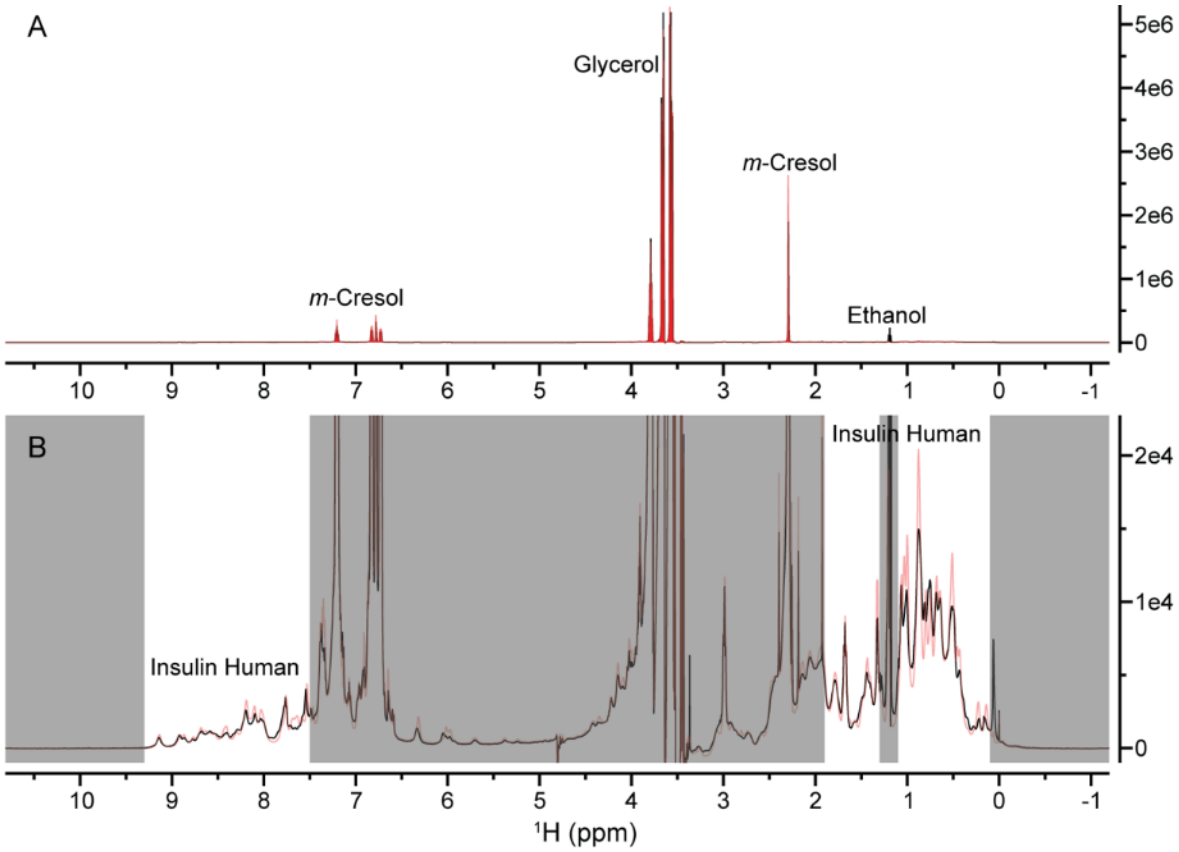


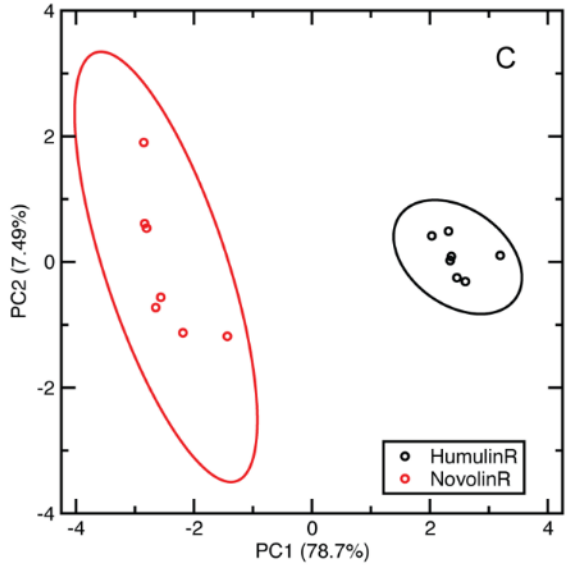
Humalog®
and
Admelog®

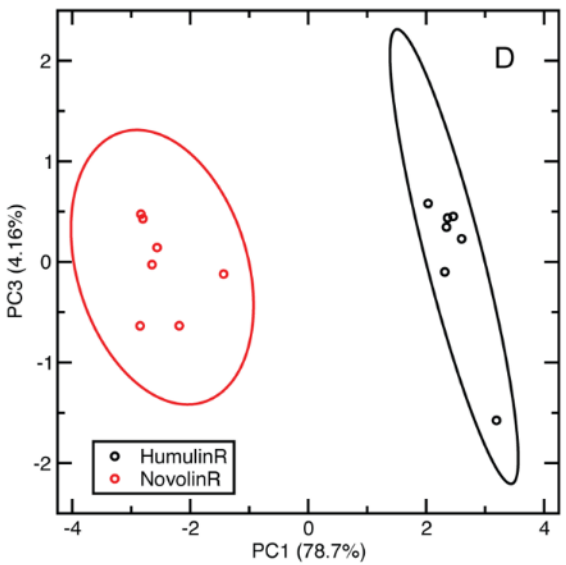




Lantus® and Basaglar®

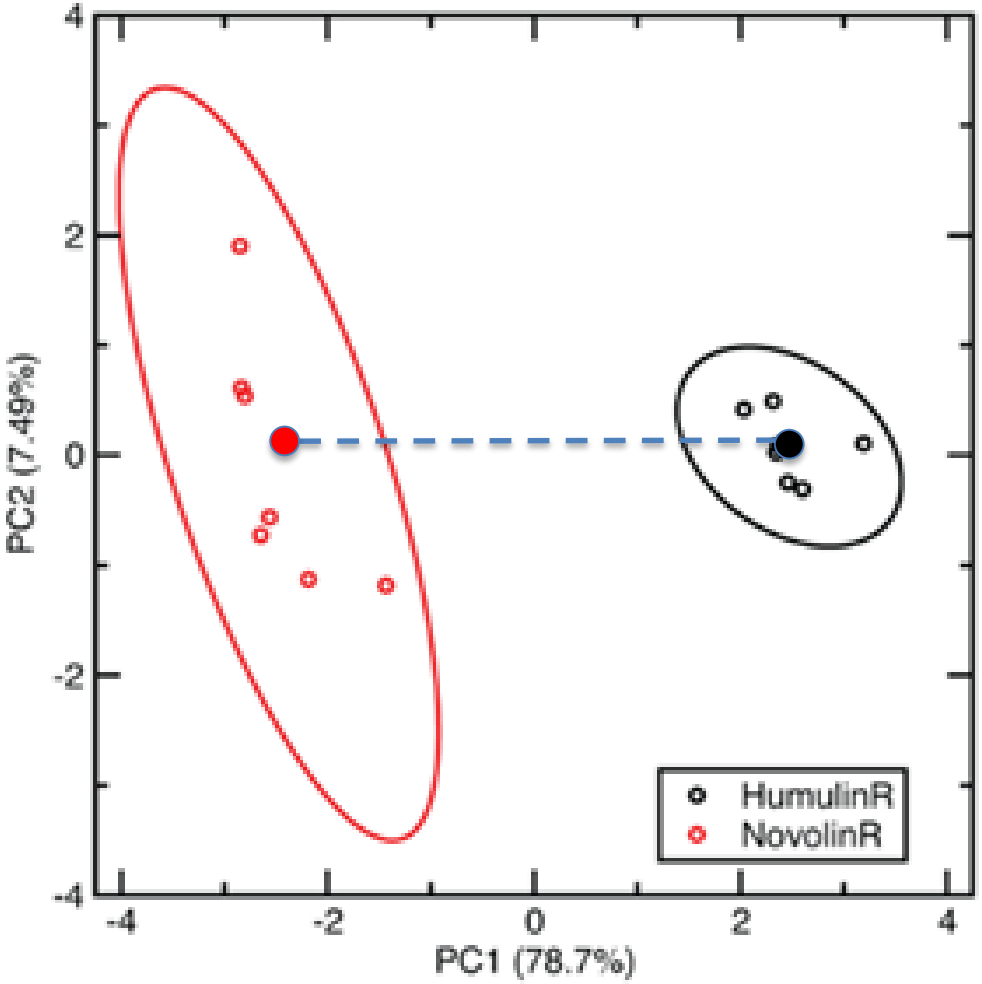






HumulinR® and NovolinR®

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)

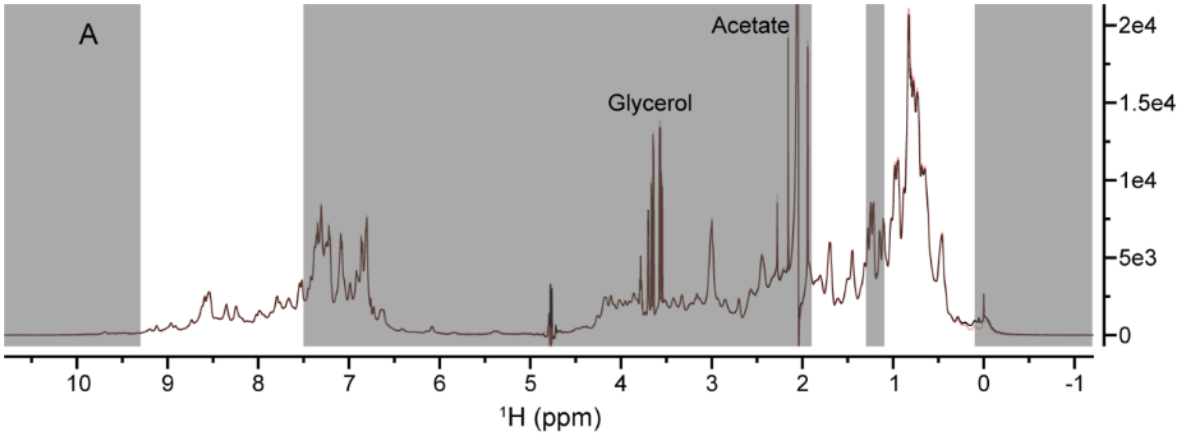
$$D_M = \mathrm{sqrt}[(\bar{Z}_H - \bar{Z}_N)S^{-1}(\bar{Z}_H - \bar{Z}_N)']$$

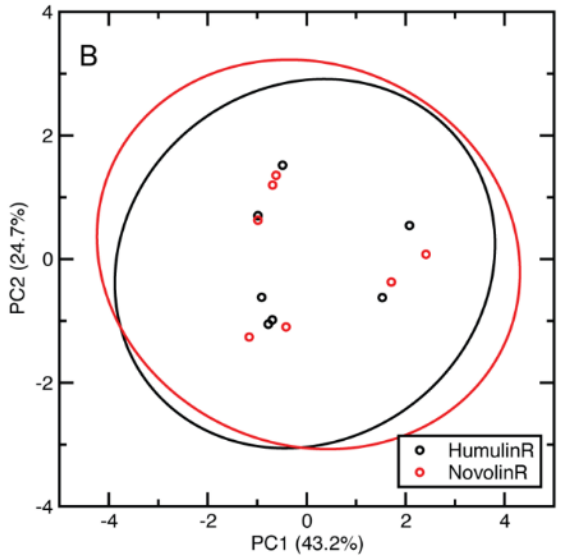
Chen K, Park J, Li F, Patil SM, Keire DA. *AAPS PharmSciTech*, 2018, 19(3):1011-1019.

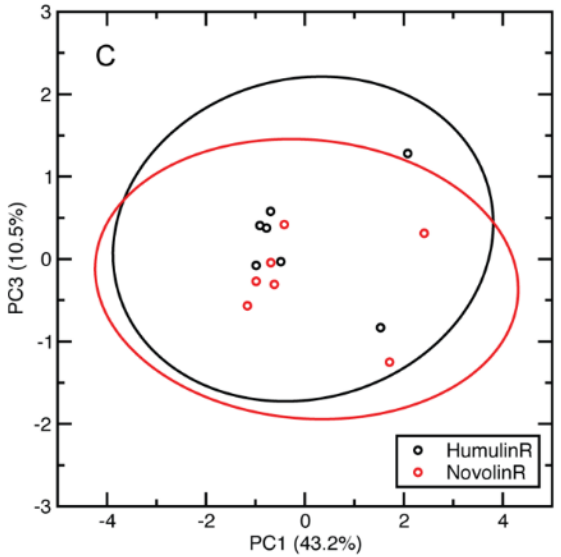
Similarity Threshold for DP

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	

Insulin Human at pH 4







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

CONCLUSIONS

- New technology for the assessment of biologics to assure the quality of such complex multi-attribute drugs is desired.
- New technology has to be shown (peer reviewed scientific literature helps) to be robust and repeatable across laboratories.
- Demonstration of enhanced sensitivity or resolution compared to “classical” methods is necessary. Sufficient examples provided by publications provide a paradigm shift.
- NMR has a potential to be a routine testing method for drug quality control and surveillance.

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Rob Brinson
Luke Abrogast
Frank Delaglio

UMBC:
Junyong Park
Feng Li

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