Analytics for complex drugs: FDA examples

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AT Europe
March 15, 2017

Complex Drugs

- Can be biologically sourced
  - Heparin from pig or cow (1939)
  - Conjugated estrogens (over 60 active ingredients) (1941)
  - Peptide drugs (*e.g., Insulin* 1982, now and in the future)
  - Protamine sulfate (1969)
  - Enoxaparin LMWH (1993 and 2010 1st generic)
  - Large protein therapeutics (now and the future)
- Can be synthetic and complex
  - Peptide drugs (now and the future)
  - Glatiramer acetate (1996 and 2015 1st generic)
  - Colloidal Iron (Feraheme 2009)

Good to have a bigger boat

Modern Analytics are a bigger boat!

- Provide information-rich data for complex drug structure and composition assessment:
  - In comparison studies extra peaks or intensity changes can indicate impurities, contaminants or structure alterations.
  - Important to establish the normal range of variability for each drug (generally no reference standards exist).
- Raise the bar for drug analysis:
  - The more unique properties measured in the most sensitive way possible, the better the characteristics of a complex drug are defined.
  - The better a drug is defined analytically, the greater the assurance of drug quality.

How do you know when your boat is big enough?

Clinically Meaningful Differences

- Aspects of drug quality known to impact the safety or efficacy of a therapeutic should be analytically assessed.
- Where impact or mechanism of action is not known as many drug characteristics as possible should be measured for clinically tested lots.
  - Drug substance characteristics (e.g., Higher Order Structure)
  - Impurity profile

Peptide Drugs

New Molecular Entities (NMEs):

- More than 60 FDA approved peptide drugs on the market.
- 140 peptide drugs in clinical trials
- Over 500 peptide drugs in preclinical development

Abbreviated New Drug Applications (ANDAs):

- Many peptide applications pending
- Most quality control methods submitted are ***HPLC-UV*** based
  - These methods may not be adequate to resolve the peptide related impurities.

Fosgerau *et al.*, Drug Discovery, 20(1), 122-128), 2015

Peptide Generics

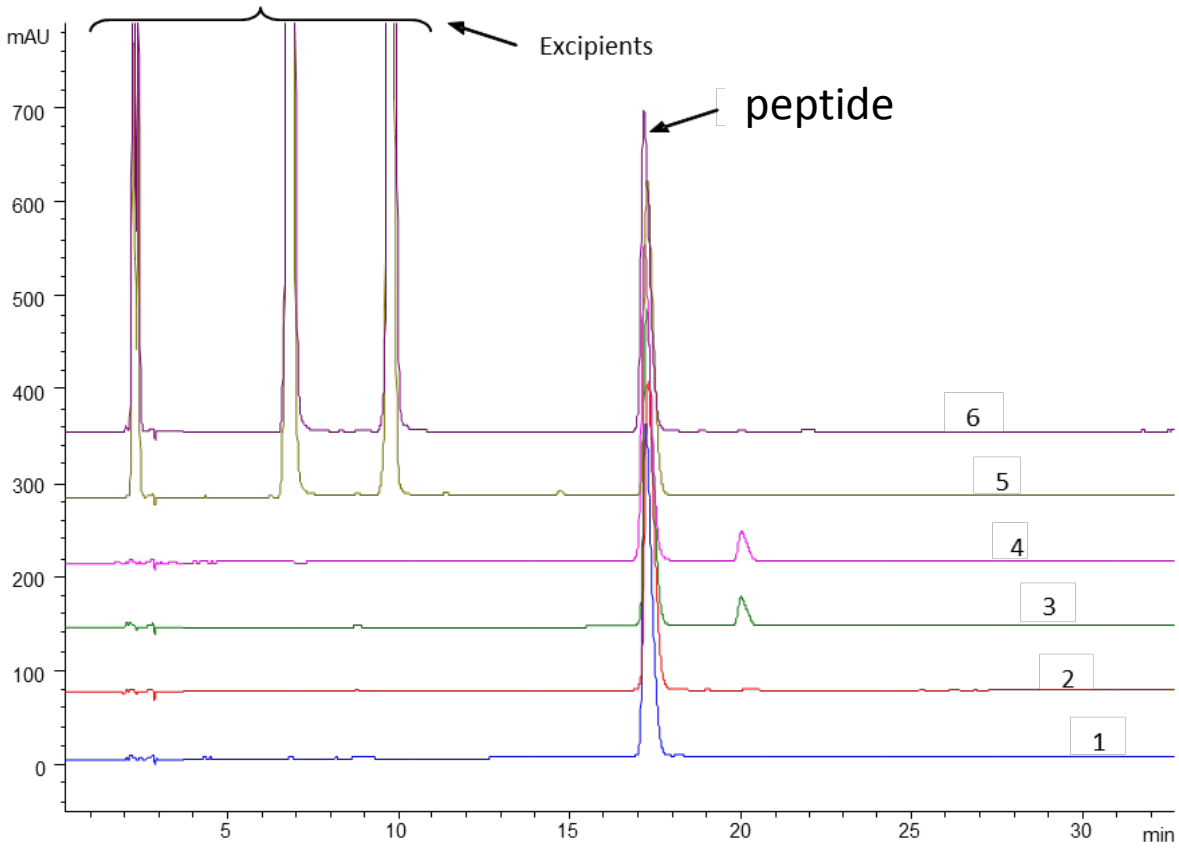
- Pharmaceutical Equivalence
  - Drug Substance Sameness
  - Same dosage form
  - Same route of administration
  - Appropriate quality (identity and purity)

Two scenarios: Synthetic RLD vs Synthetic ANDA
Recombinant RLD vs Synthetic ANDA

Times have changed.

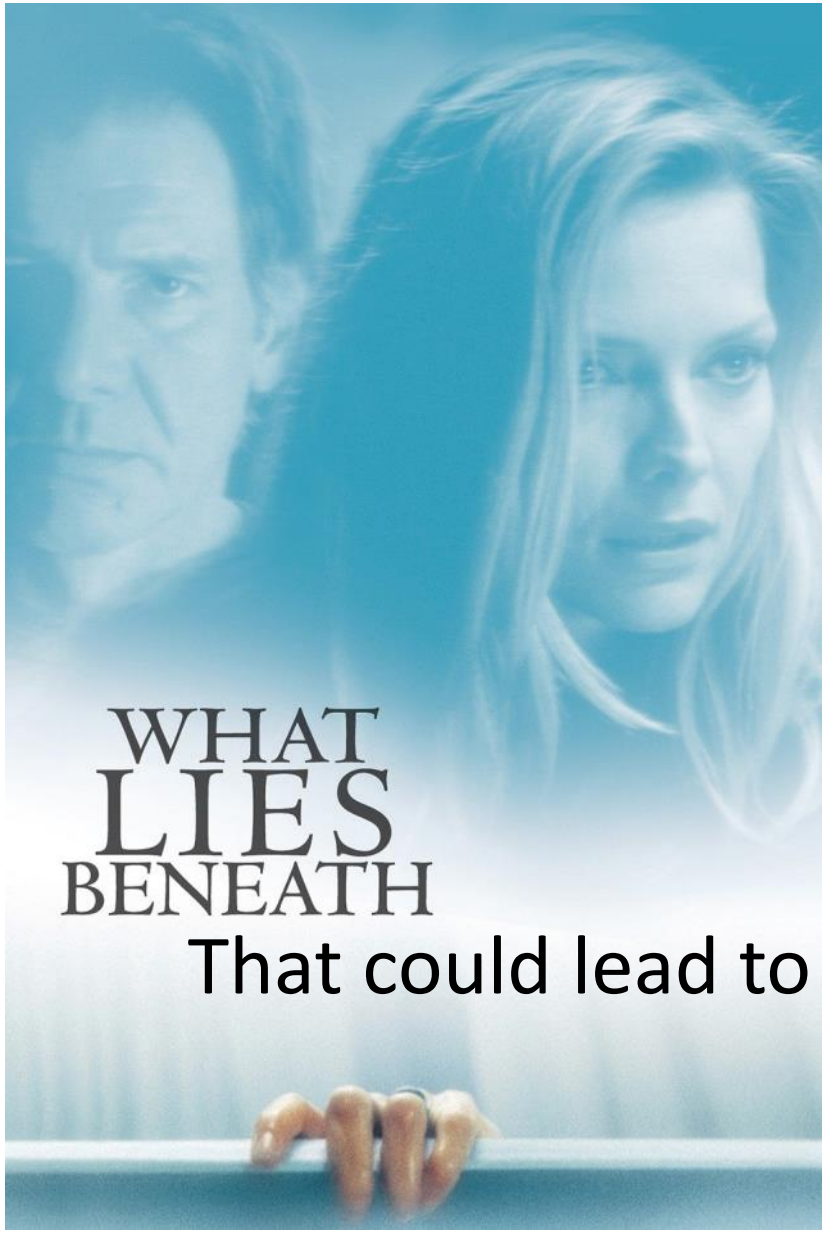
- Human recombinant insulin was approved in 1982.
- Since then peptide synthesis methods have improved allowing most peptides to be made economically by chemical synthesis.
- Analytical technology has changed since many of the reference licensed drugs were approved.

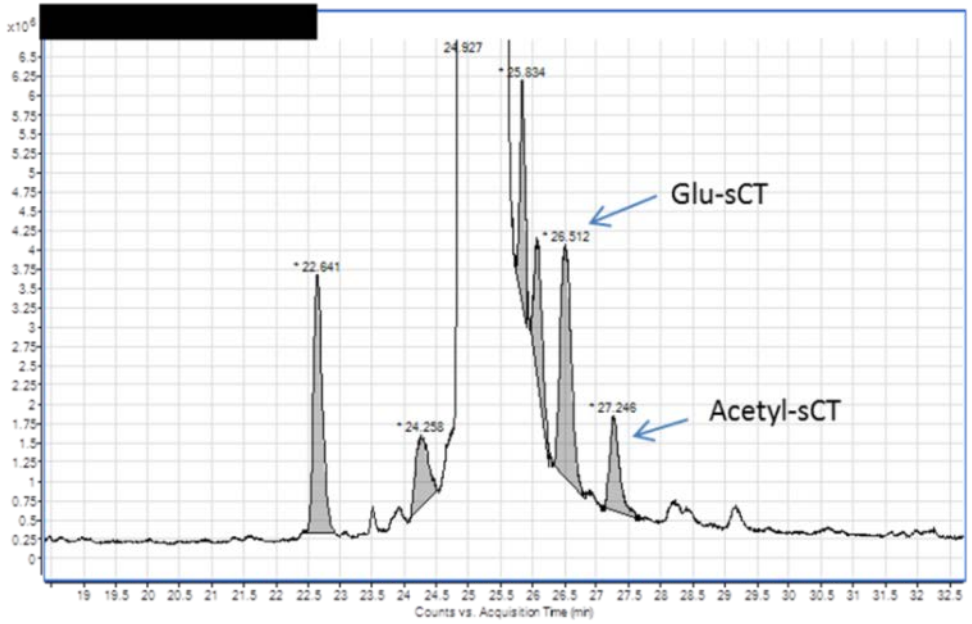
Peptide Drug HPLC-UV



USP HPLC assay.

1. Manufacturer 1, Lot# 1
2. Manufacturer 2, Lot# 1
3. Manufacturer 3, Lot# 1
4. Manufacturer 3, Lot# 2
5. Manufacturer 4, Lot# 1
6. Manufacturer 4, Lot# 2

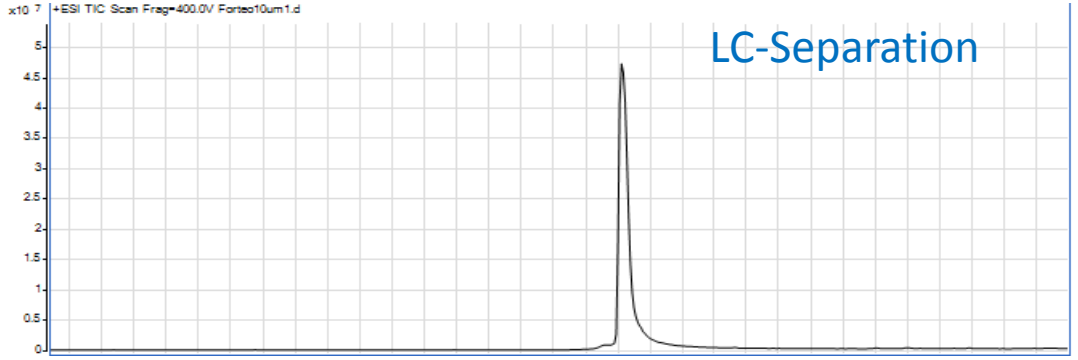


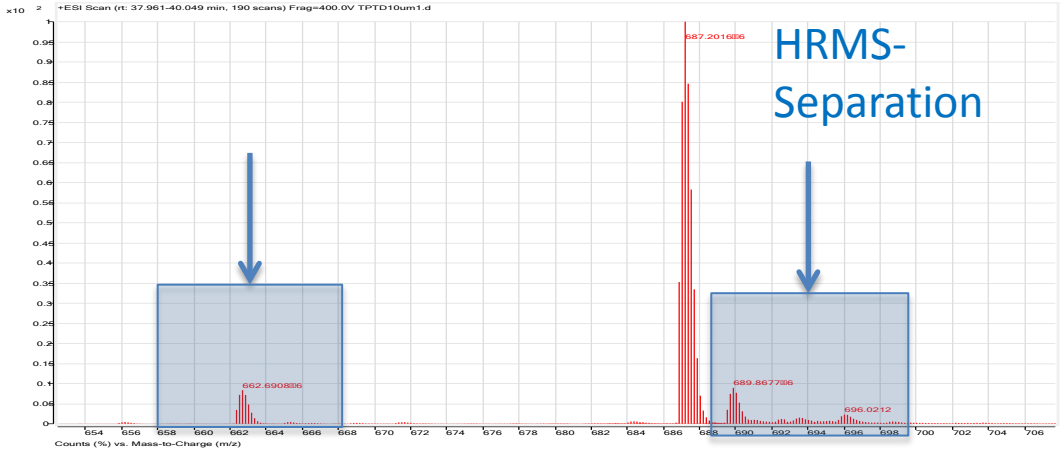


That could lead to immunogenicity risk?

LC-MS is a Ghostbuster!

-high sensitivity for peptide ID and quant.





Immunogenicity factors

- Active Ingredient
- Route of administration
- Dose and administration
- Patient
- Peptide related impurities
- Aggregates
- Excipients
- Leachables

What lies beneath.

Lee *et al.,* AAPS J, 13(1), 14-19, 2011
Rosenberg, AAPS J. 8, E501–507, 2006

Common impurities in recombinant or synthetic peptide or drugs

rDNA derived drug	Synthetic Peptide Drugs
Modifications to C, M, H, K, W: oxidation, reduction, deamidation, pyro-Glu etc…	Modifications to C, M, H, K, W: oxidation, reduction, deamidation
Fragmentation	Incomplete removal of protection groups: tBu, FMOC, tBOC etc…
Aggregation	Amino Acid Racemization: D-conformer instead of L-conformer
Sequence variants	Amino Acid deletions
Host Cell Proteins	Amino Acid insertions

Zeng et al., AAPS J, 17(3), 643-651, 2015
Eon-Duval et al., Biotechnol Prog, 28(3), 608-622, 2012
D'Hondt et al., JPBA, 101, 2-30, 2014

Peptide Impurities: Known Risks

- Host Cell Proteins in rDNA derived peptides[1]
- Residual tBu groups[2]
- D-form AAs[3]
- Peptide contaminants from other syntheses[4,5]

1 Haile *et al.,* PLOS One, April 2015, 1-17, 2015
2 Reid *et al.,* Immunology, 144, 495-505, 2014
3 Van Regenmortel and Muller, Curr Opin Biotech., 9, 377-382, 1998
4 Brezar *et al.,* PLOS One, 6(12), 1-9, 2011
5 Currier *et al.,* Clin. Vac. Immuno., 15(5), 267-276, 2008

Salmon Calcitonin

Species	Sequence	Relative Activity
Salmon Calcitonin	CSNLSTCVLG KLSQELHKLQ TYPRTNTGNG TP-Amide	~25
Human Calcitonin	- G- - - - -M-- TYT- -DFN- - FH- F-Q- A I- V-A-Amide	1

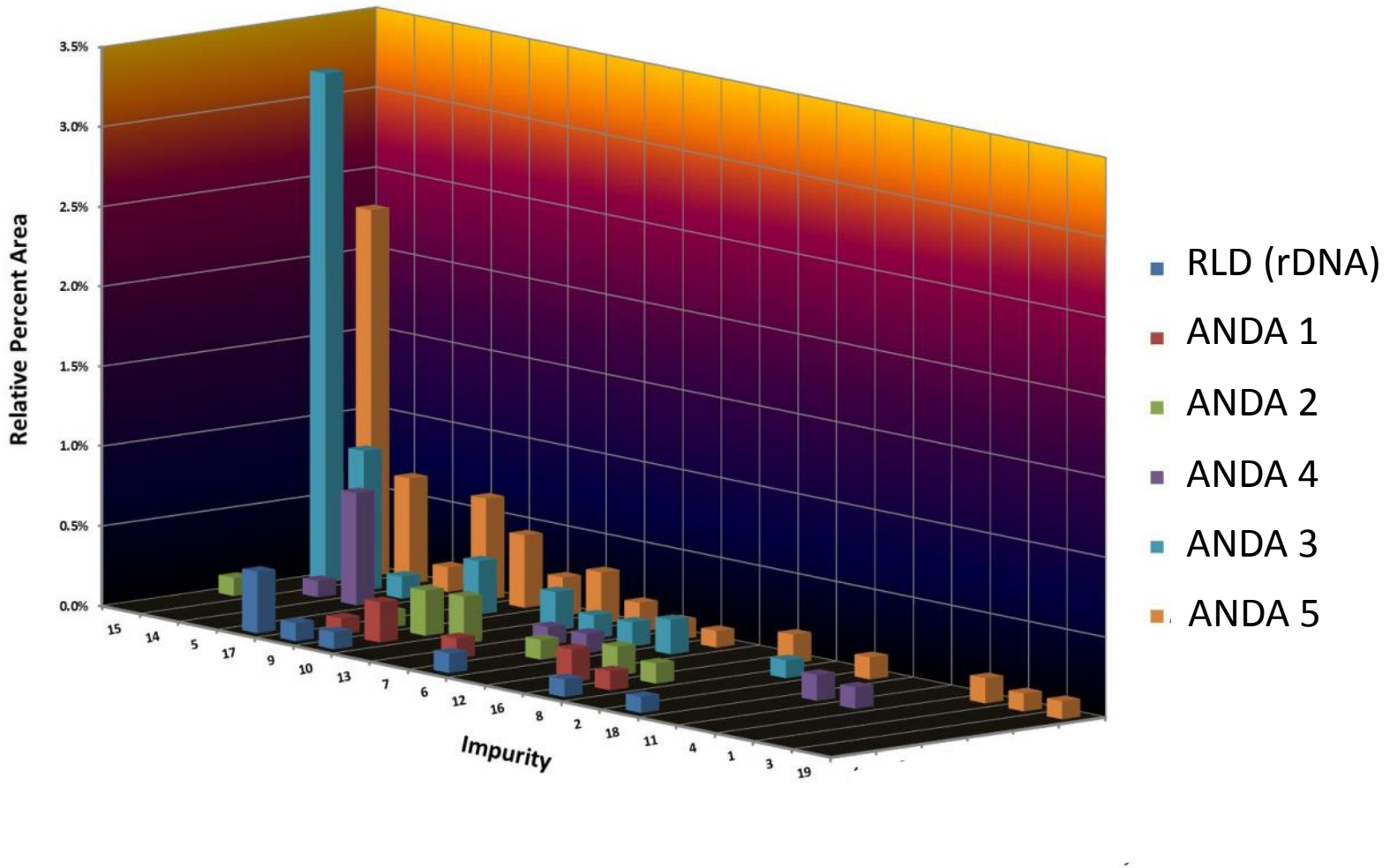
- 50% sequence homology between human and salmon form.
- Formation of antibodies to the drug substance is common (40-70%).
- However, therapeutic efficacy was not lost in most seropositive patients.
- Based on available evidence the immune response is to a specific salmon sequence.

Kozono, *et al.,* Endo, 131, 1412-1425, 1992
Grauer, *et al.,* AM. J. Med., 95, 439-442, 1993

Calcitonin-Salmon Peptide Impurities

- 15 batches from 5 different firms were analyzed.
- Over 130 peptide impurities were detected using LC-MS.
- Differences were observed between synthetic and rDNA products.

Peptide Impurity Profiles



LC-HRMS vs USP LC-UV

- For the calcitonin RLD LC-HRMS identified 12 impurities for a total of 2.6% (Area%).
- When the same sample was analyzed by the USP HPLC-UV method, 6 impurities were observed with a 2.0% total.
- Detection limits for the 2 identified peptide impurities were below 0.1% (Area %) by LC-HRMS.

Zeng et al., AAPS J, 17(3), 643-651, 2015

Protamine Sulfate

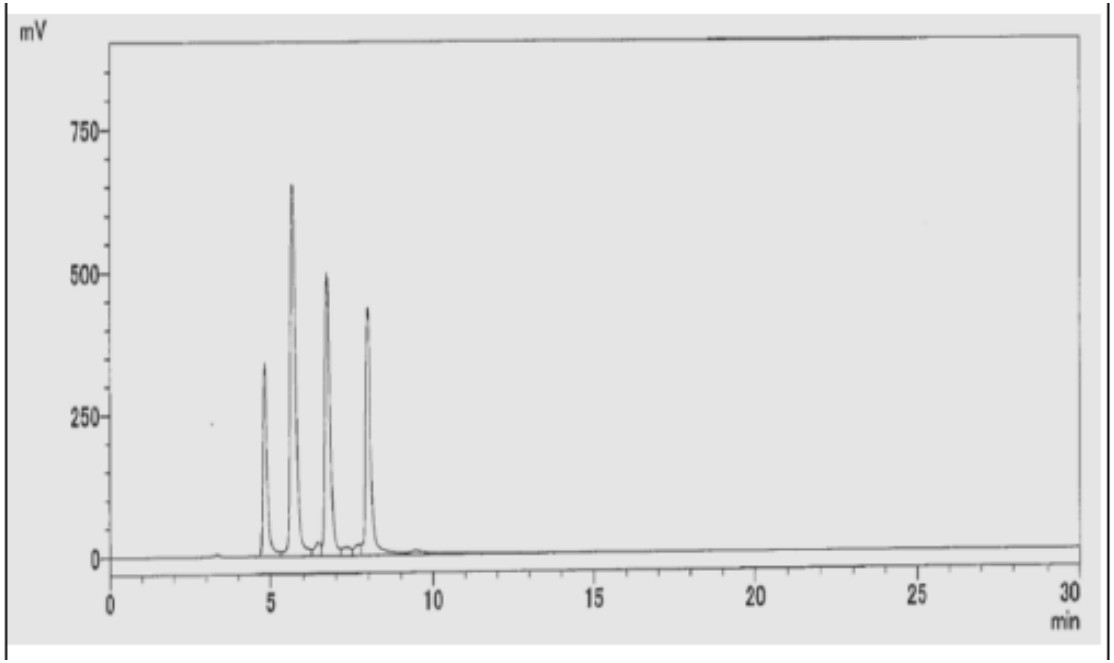
Fish	Amino Acid Sequence
Chum salmon #1	PRRRRRSSS RPIRRRRRPR ASRRRRR-GG RRRR 21
Chum salmon #2	PRRRR-SSR RPVRRRRRPR VSRRRRRRGG RRRR 22
Chum salmon #3	PRRRR-SSS RPVRRRRRPR VSRRRRRRGG RRRR 21
Chum salmon #4	PRRRR-ASR R-IRRRRRPR VSRRRRR-GG RRRR 21

- Clinically used to neutralize heparin sodium activity post surgery.
- On the WHO list of Essential Drugs.
- The high similarity of the peptide sequences makes them difficult to resolve using HPLC.
- Because of that we have performed MS and NMR studies for improved methods for assay, identity and purity.

Gucinski A.C. and Boyne M.T. 2nd, "Identification of site-specific heterogeneity in peptide drugs using intact mass spectrometry with electron transfer dissociation", *Rapid Commun. Mass Spectrom.*, 28(15), 1757-1763, **(2014)**
Gucinski A.C., Boyne M.T. 2nd and Keire D.A., "Modern analytics for naturally derived complex drug substances: NMR and MS tests for protamine sulfate from chum salmon", *Anal. Bioanal. Chem.*, 407(3), 749-759, **(2015)**

Protamine Sulfate USP HPLC assay

- Typical chromatogram provided with US Pharmacopeia protamine sulfate reference standard



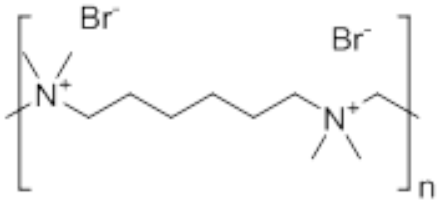
Buffer A: 0.3 M Phosphate pH 1.8
Buffer B: A + 6.5 v/v ACN
UV 214 nm detection, L1 4.6 x 250

Time (min)	Solution A (%)	Solution B (%)
0	85	15
15	55	45
25	55	45
30	85	15

What about Capillary Electrophoresis?

What is known?

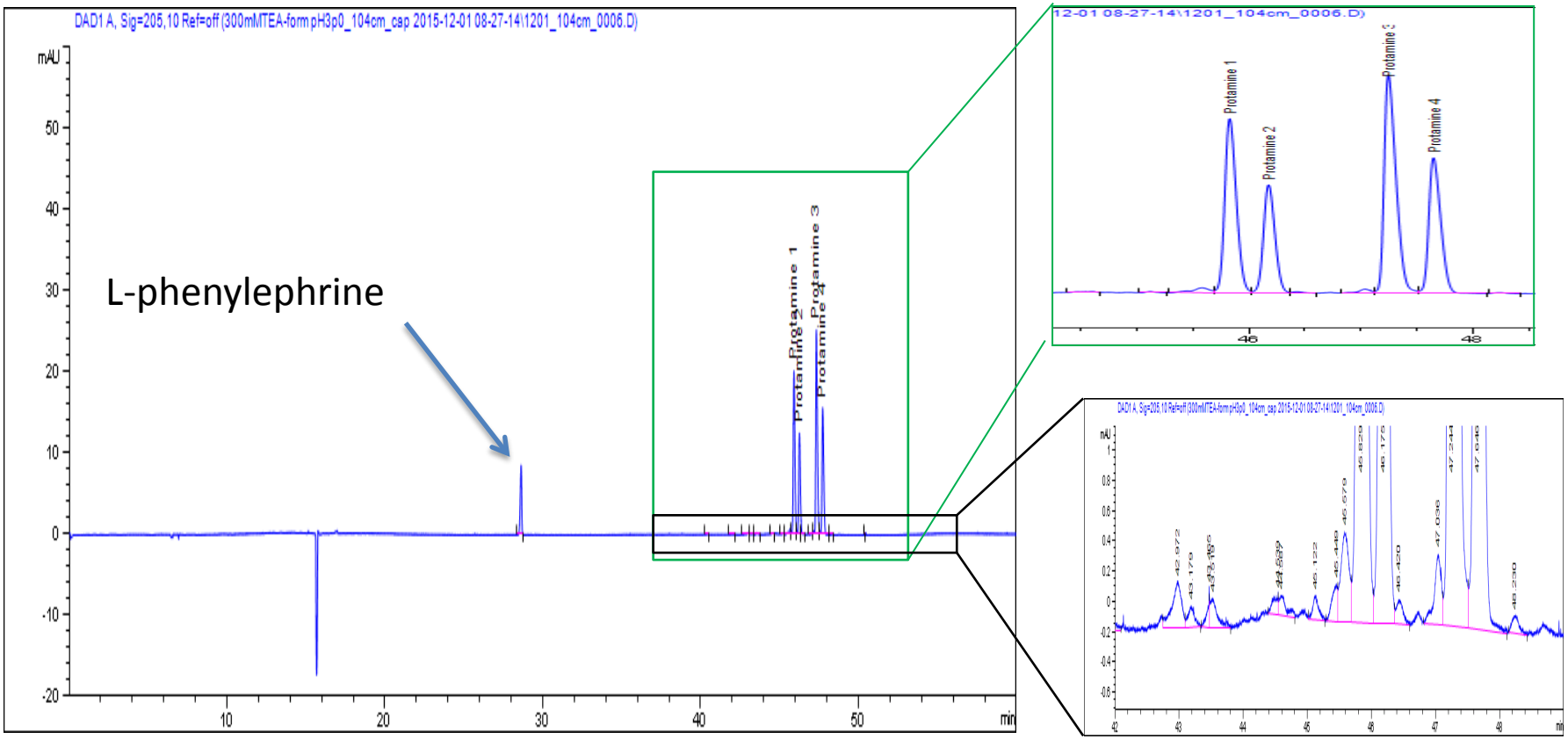
- ***One*** paper identified with a published capillary zone electrophoresis (CZE) method for human protamine.
  - The interaction between the negatively charged capillary surface and ***cationic*** analytes is bad for separation.
- Many papers on analysis of basic proteins by CZE, common approaches.
  - Analysis at acidic pH (mostly pH 3 to 5)
  - Capillaries modified with permanent coatings
  - Background electrolyte modified with compounds yielding reversed electroosmotic flow (e.g., triethylammonium formate)
  - Capillaries modified dynamically with compounds in the background electrolyte yielding reversed electroosmotic flow (towards the anode)



Polybrene (PB)
(hexadimethrine bromide)

Capillary Zone Electrophoresis with dynamic coating and EOF buffer

Capillary prep: polybrene coating
Assay: triethylammonium formate buffer
Between injections: flush and recoat
Desired MS compatible buffer
Dynamic coating with polybrene



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®)

Round robin study on the comparability of NMR spectral 'fingerprints' obtained using standardized NMR experiments

4 Sites in North America and Europe

FDA; Health-Canada; MPA-Sweden; NIST

4 Fields – Six spectrometers

500, 600, 700 and 900 MHz

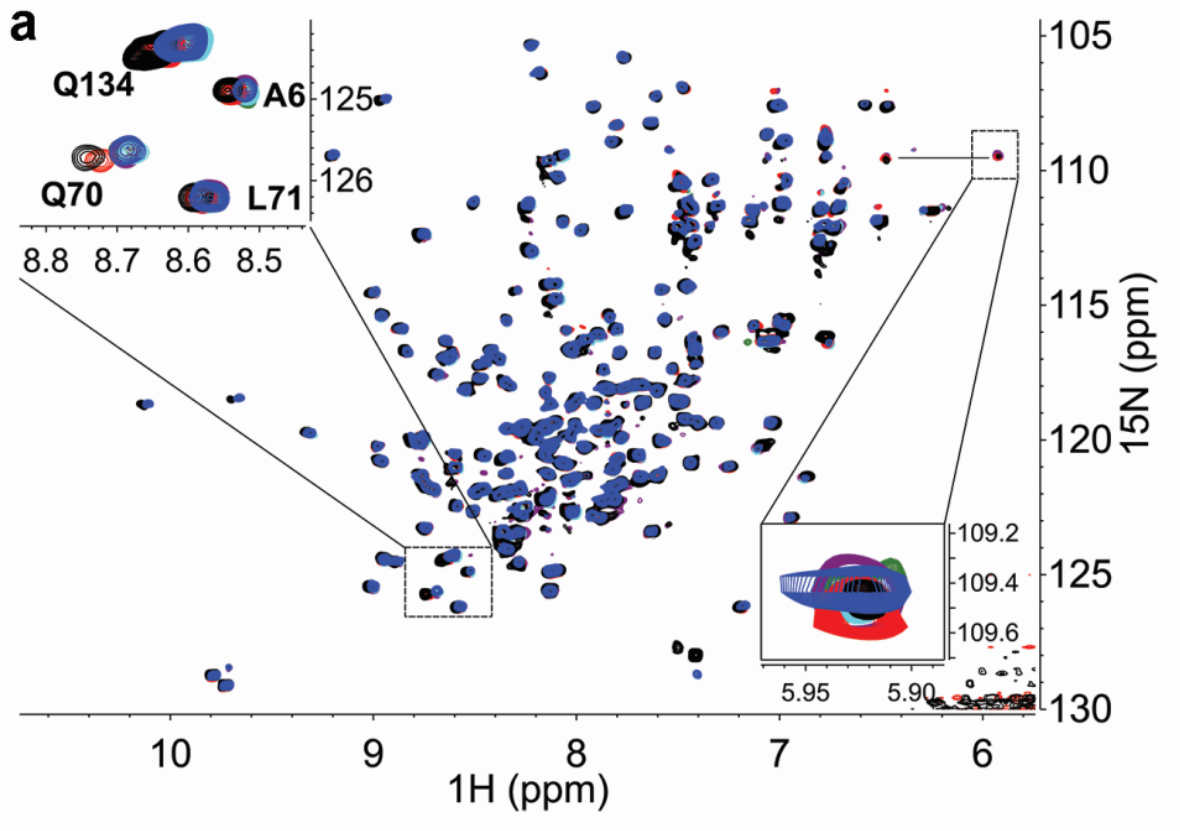
Different Instrument vintages

2 Vendors

Bruker Biospin, Varian/Agilent

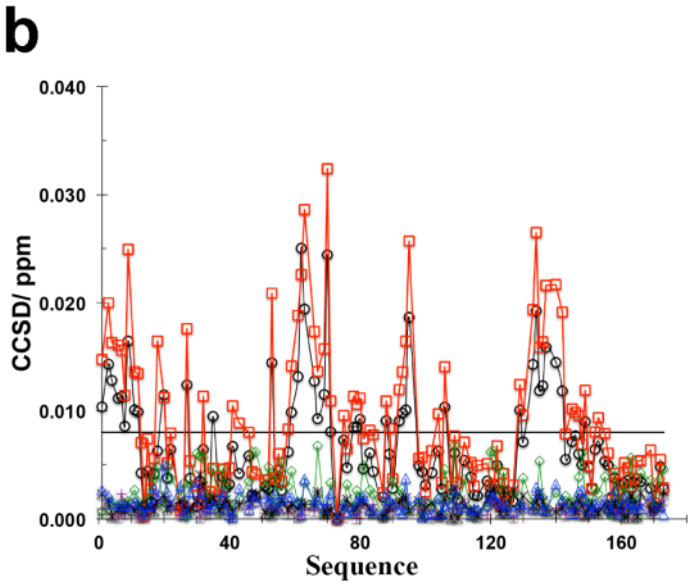
Overlay of the $^{1}H,^{15}N$-HSQC 2D maps for G-CSF at 500 MHz and 25°C

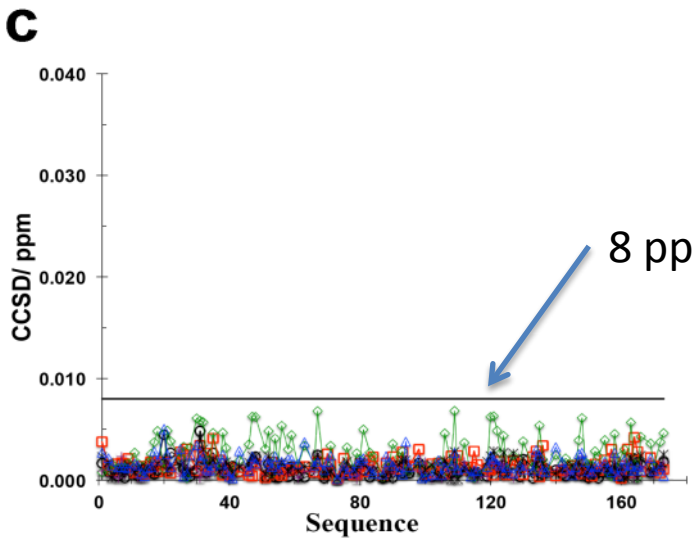
46 hrs on 900
89 hrs on 500
S/N > 10
For designated Signals.



Health Canada 600 and 700 data is shifted for some of the signals in the cross laboratory comparison?

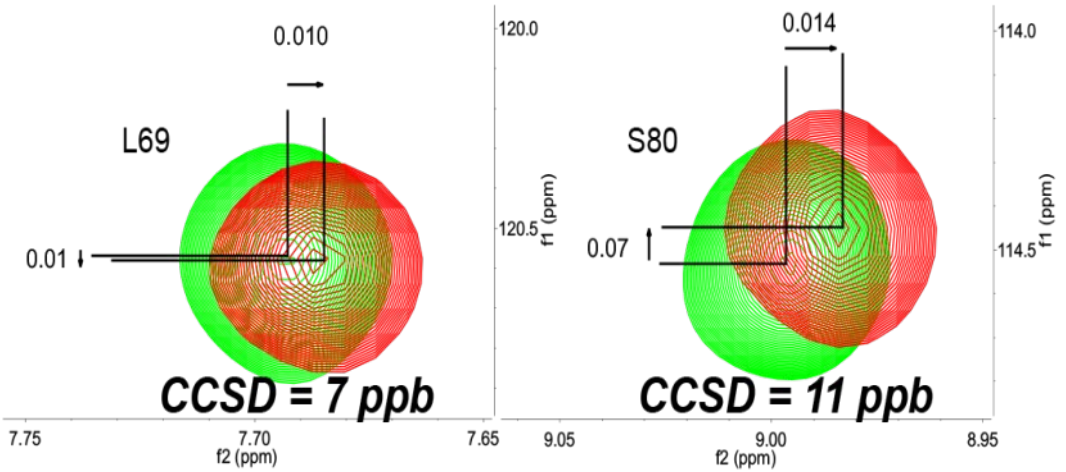
Very Precise!





8 ppb

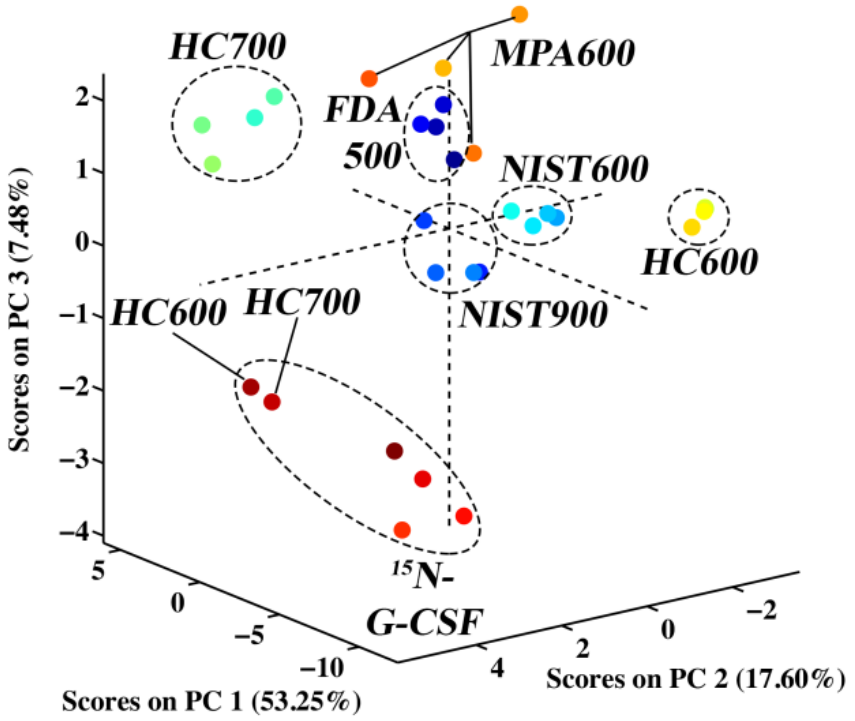
Two instruments turned out to be out of calibration for probe air temperate



With calibration for probe air temperate

Chemometrics

- 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 H., et al. "Precision and Robustness of 2D-NMR for Structure Assessment of Filgrastim Biosimilars," *Nature Biotechnology*, 34(2), 139-141, (2016).

Acknowledgements:

OTR/DPA:

Michael Hadwiger

Kui Zeng

Ilan Geerlof-Vidavsky

Sarah Rogstad

NMR-RR

Robert Brinson (IBBR-NIST)

Luke Abrogast (IBBR-NIST)

John Marino (IBBR-NIST)

Derek Hodgson (Health-Canada)

Yves Aubin (Health-Canada)

Houman Ghasriani (Health Canada)

Ian McEwen (MPA-Sweden)

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Questions?