Agilent's Solutions for:

Biosimilars & Antibody Drug Conjugates

Gurmil Gendeh, Ph.D.
Biopharma & Biosimilars Markets

Life Sciences Group
Agilent Technologies

Outline

- Biopharma Market, Workflows & Analytical Challenges
- Biosimilars
  - Definitions & Regulations
  - How similar is similar enough
  - Case study: Comparability data between a biosimilar and its innovator reference
- ADCs
  - Rational
  - Heterogeneity in ADC
  - Key quality attributes and analytical methods for ADCs
- Summary

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Traditional Pharma business model is changing

It's a mAb!

It's a large Pharma!

HEALTH

New melanoma drug boosts survival time

By Marilynn Marchione
ASSOCIATED PRESS

CHICAGO — Researchers have scored the first big win against melanoma, the deadliest form of skin cancer. An experimental drug significantly improved survival in a major study of people with very advanced disease.

The results, reported Saturday at a cancer conference, left doctors elated.

"We have not had any therapy that has prolonged survival" until now, said Dr. Lynn Schuchter of the Abramson Cancer Center at the University of Pennsylvania, a skin cancer specialist with no role in the study or ties to the drug's maker.

The drug, ipilimumab, works by helping the immune system fight tumors. The federal Food and Drug Administration has pledged a quick review, and doctors think the drug could be available by the end of this year.

"People are going to have a lot of hope and want this drug, and it's not on their doctors' shelves," although some may be able to get it through special programs directly from its maker, Bristol-Myers Squibb Co., Schuchter said.

Melanoma is the most serious form of skin cancer. Last year in the United States, there were about 68,720

Stephen J. Boitano / Associated Press 2002

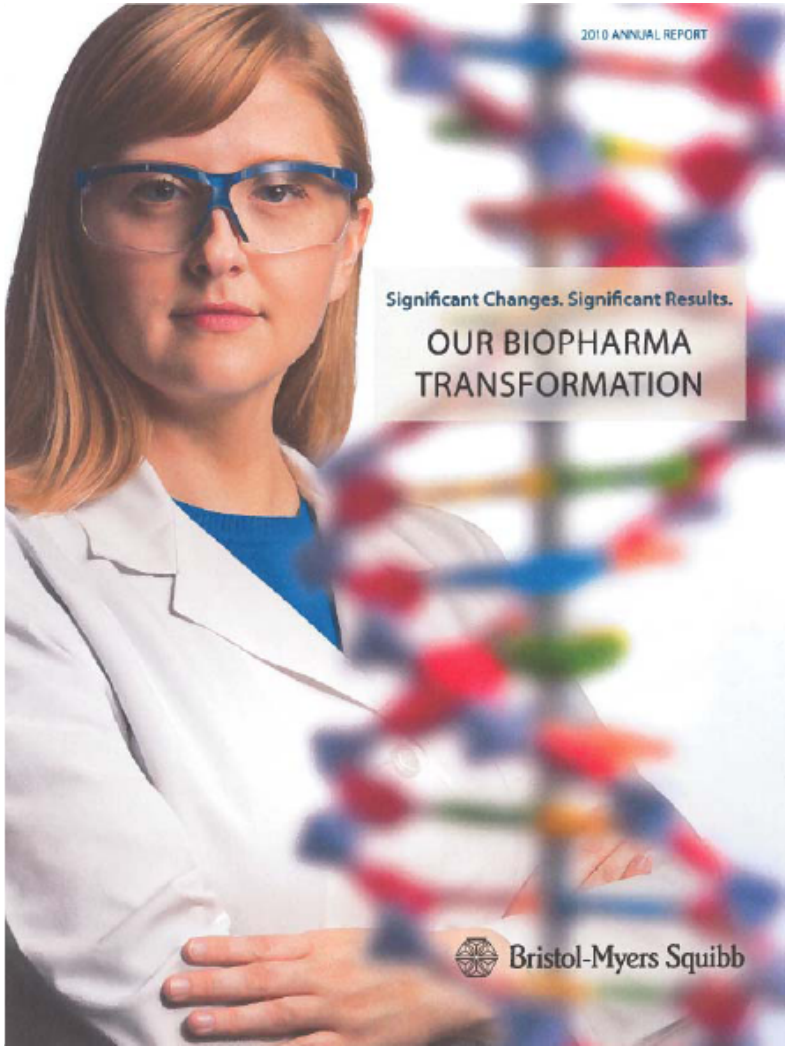
Sen. John McCain, R-Ariz., had a melanoma removed from his nose.

immune-stimulating treatment, or the immune-stimulating treatment alone.

After two years, 24 percent of those given the drug alone or in combination were alive, versus 14 percent of those given just the immune-stimulating treatment.

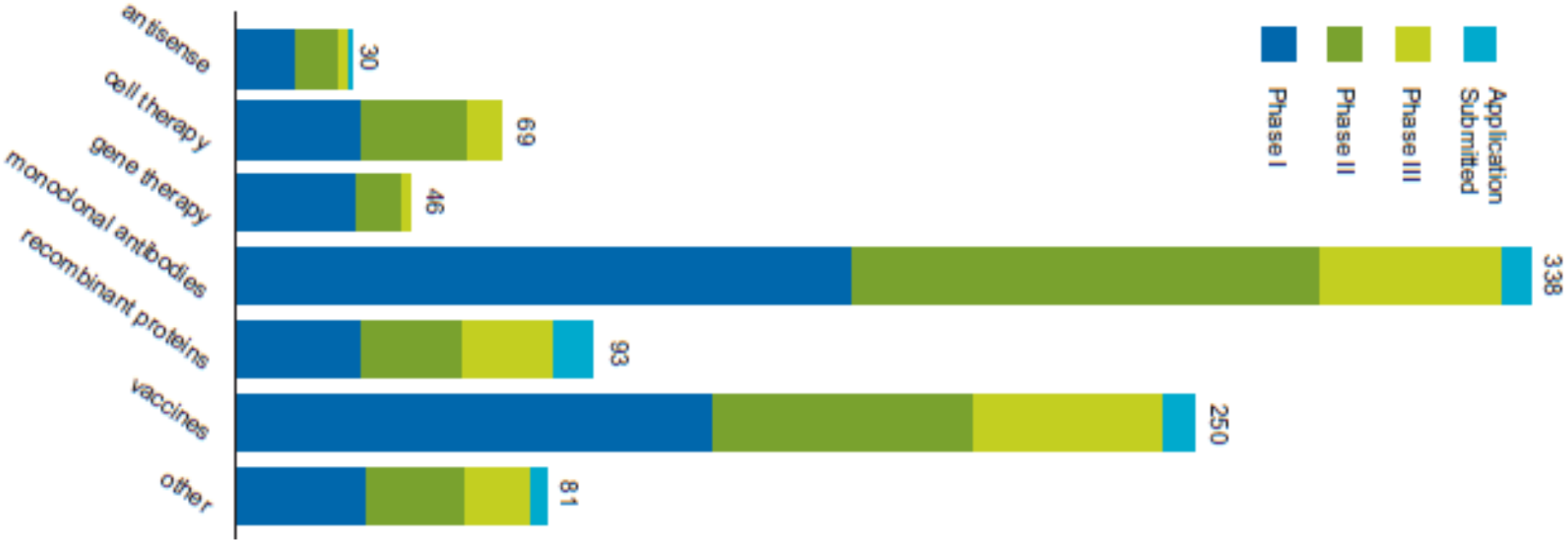
Average survival was 10 months with ipilimumab versus just over 6

Big Pharma – becoming Big Biopharma



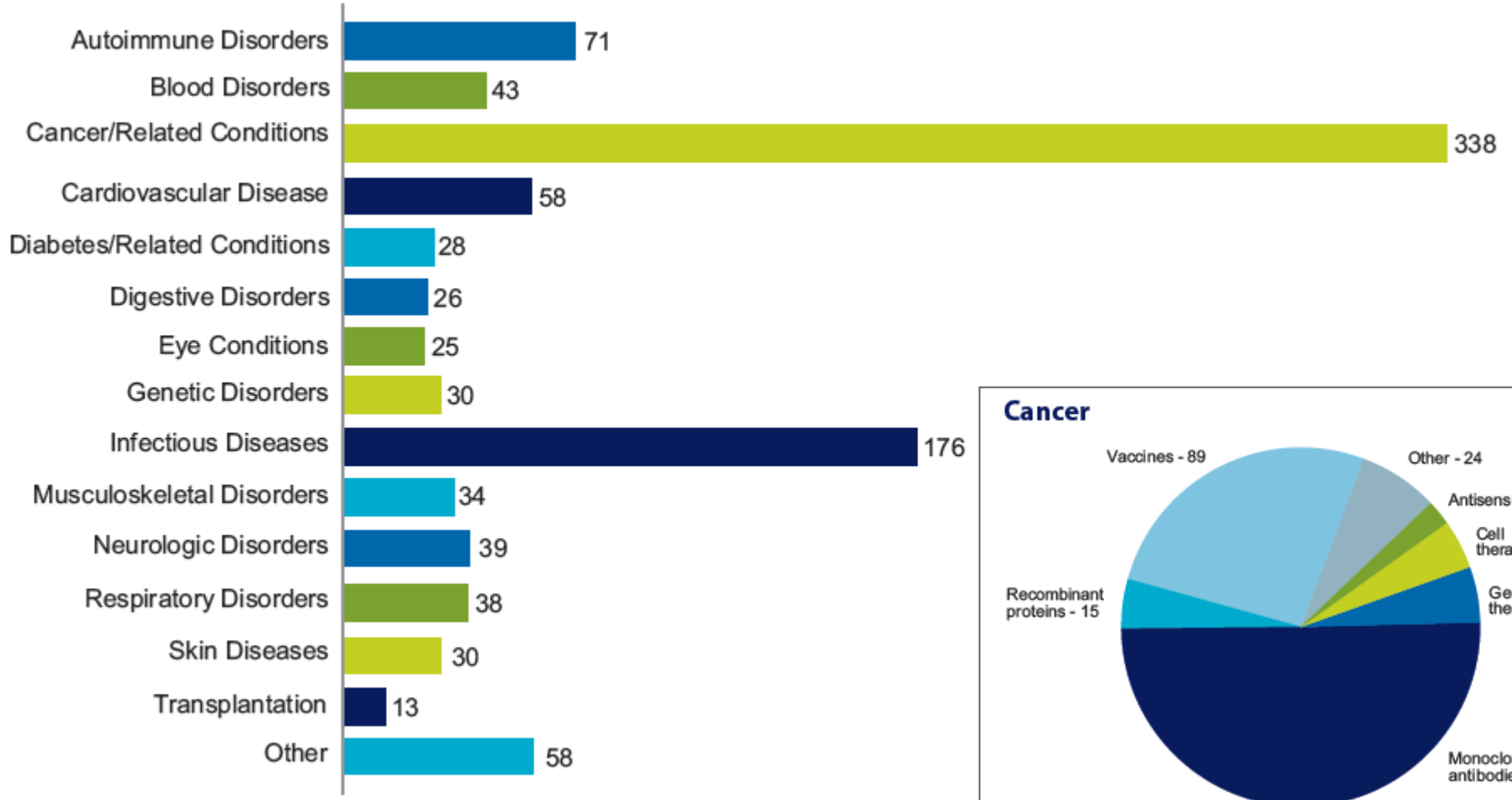
Biologics in development by product category and development phase

mAb Dominates – 3 out of 4 Protein Therapeutics are mAbs



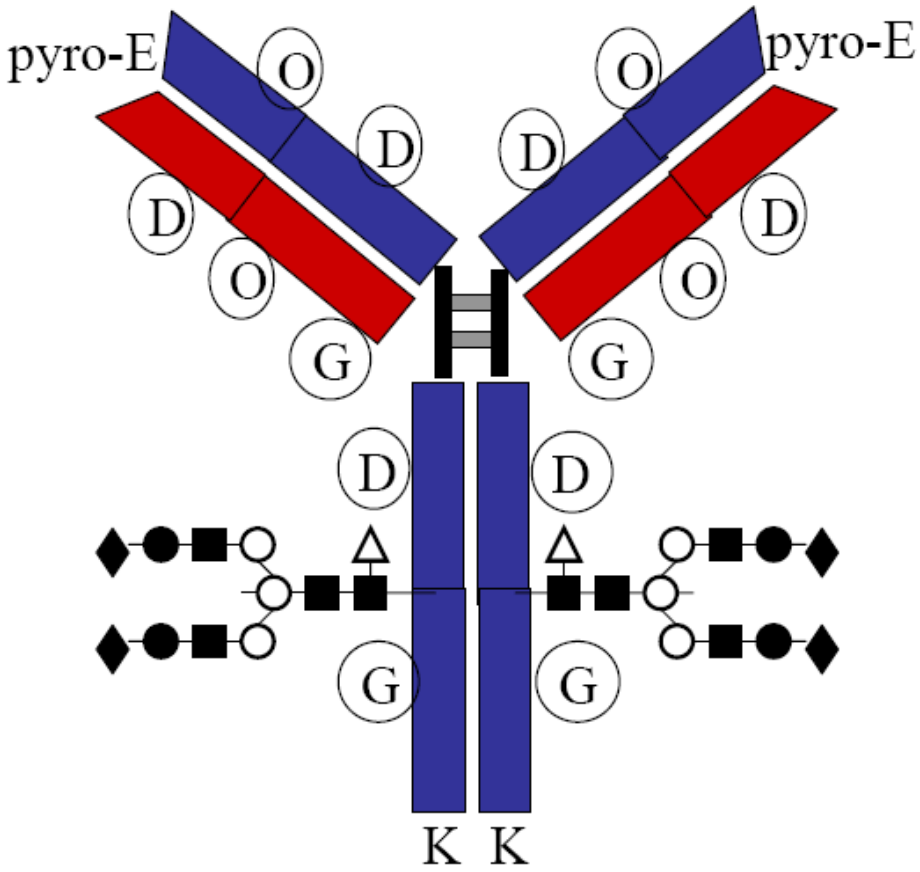
Pharma 2013 Report, Medicines in Development – Biologics

Biologics medicines – by Therapeutic categories
mAbs for Cancer therapeutics dominates



Pharma 2013 Report, Medicines in Development – Biologics

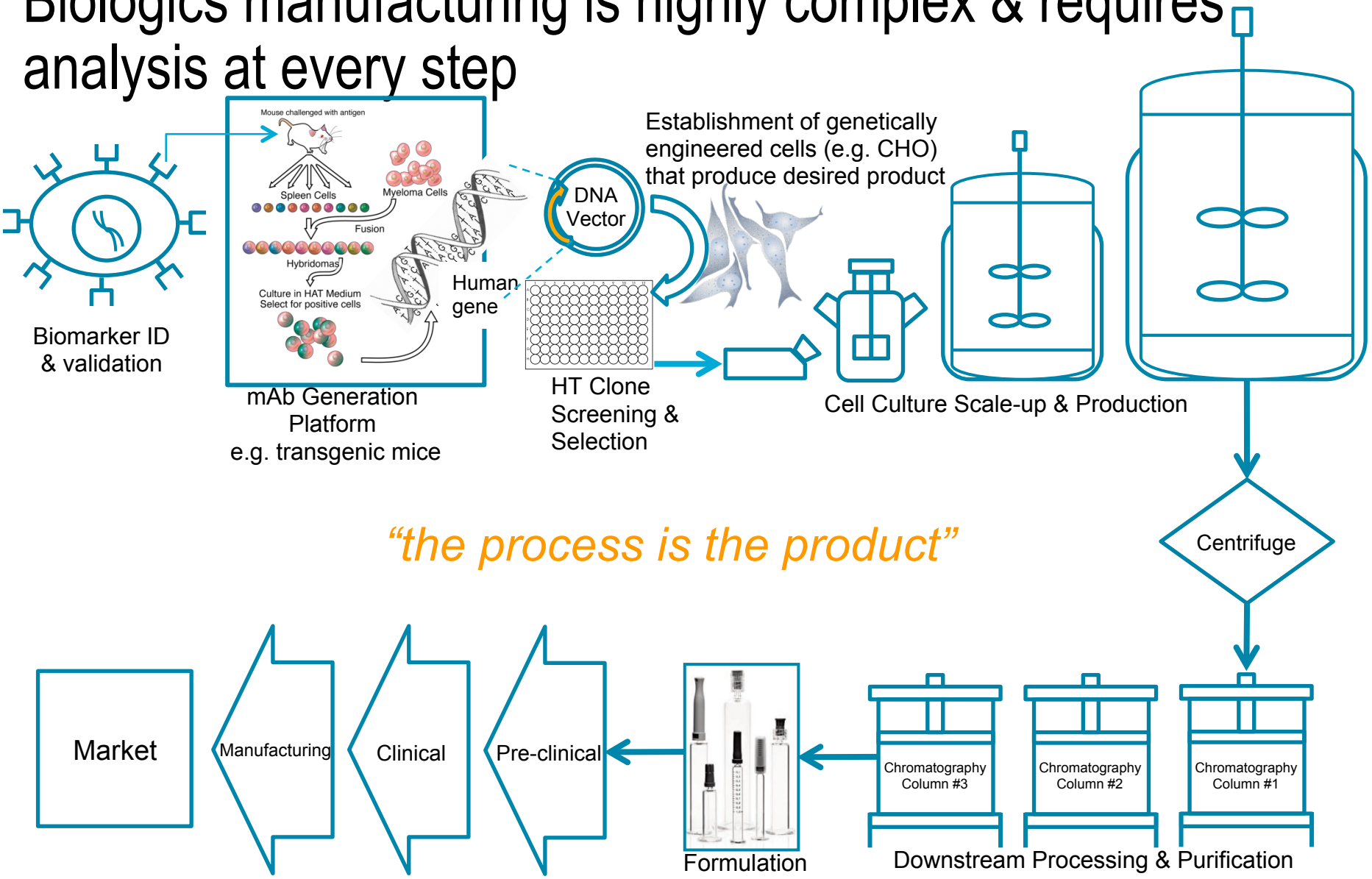
Attributes & Combinatorics



- Pyro-Glu (2)
- Deamidation (3 x 2)
- Methionine oxidation (2 x 2)
- Glycation (2 x 2)
- High mannose, G0, G1, G1, G2 (5)
- Sialylation (5)
- C-term Lys (2)

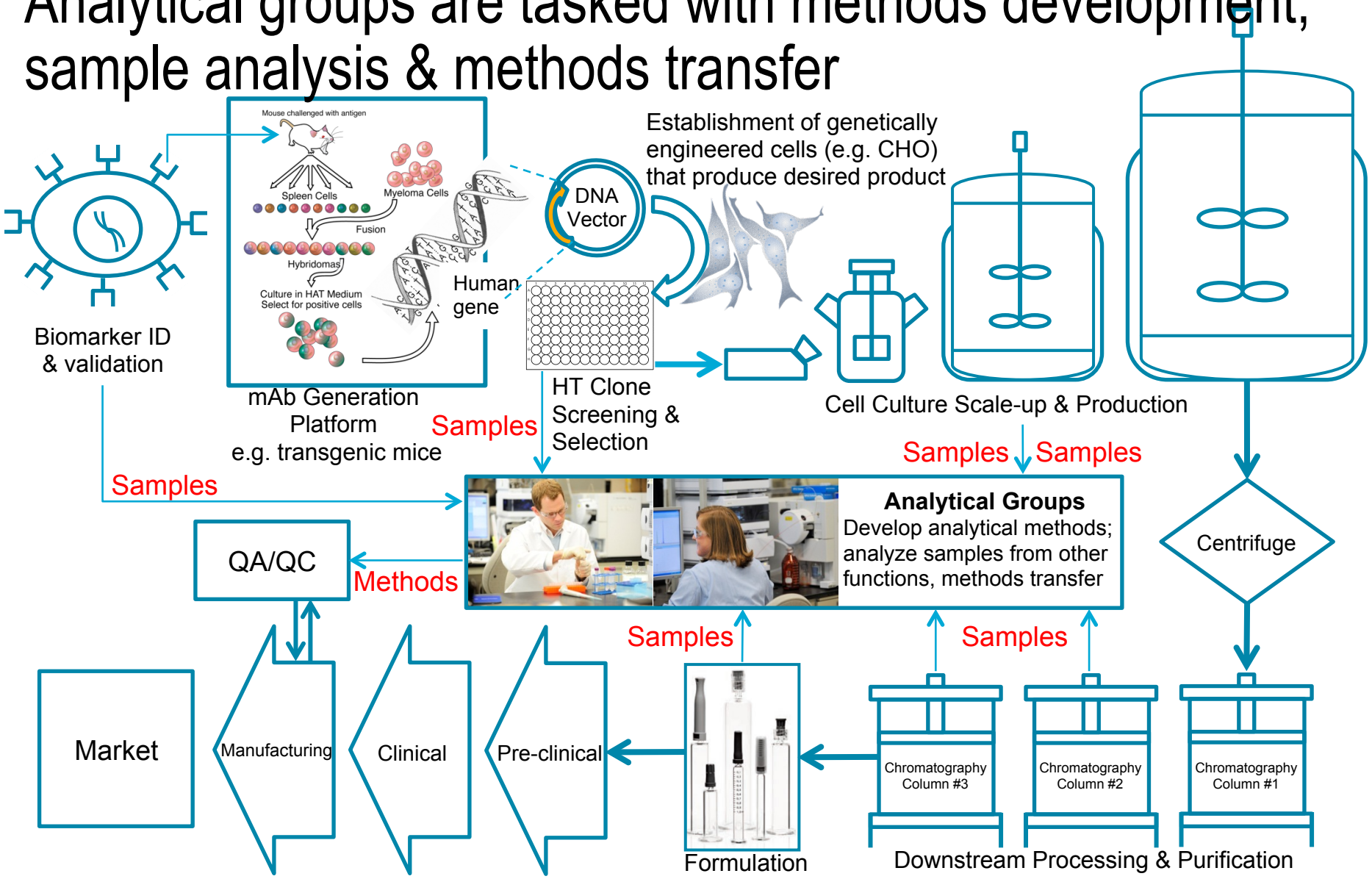
- **$(9600)^2 \approx 10^8$**
- **2 x 6 x 4 x 4 x 5 x 5 x 2 = 9600**

Biologics manufacturing is highly complex & requires analysis at every step



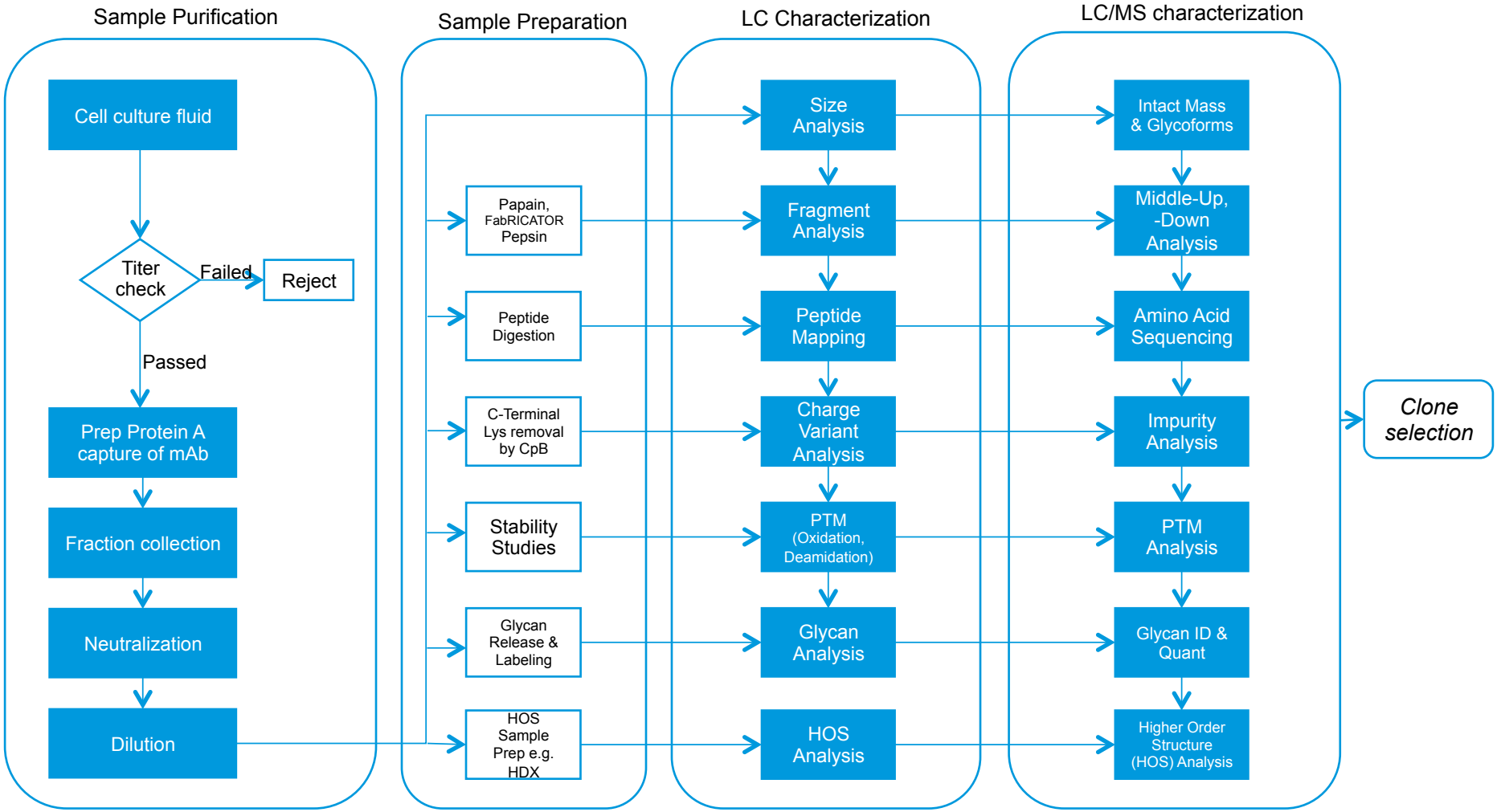
"the process is the product"

Analytical groups are tasked with methods development, sample analysis & methods transfer



Typical workflows in biopharma analytical labs — from cell culture harvest to clone selection

Samples from cell culture & purification process development, formulation, stability studies, QA/QC



New trends in Biopharma presents new analytical challenges

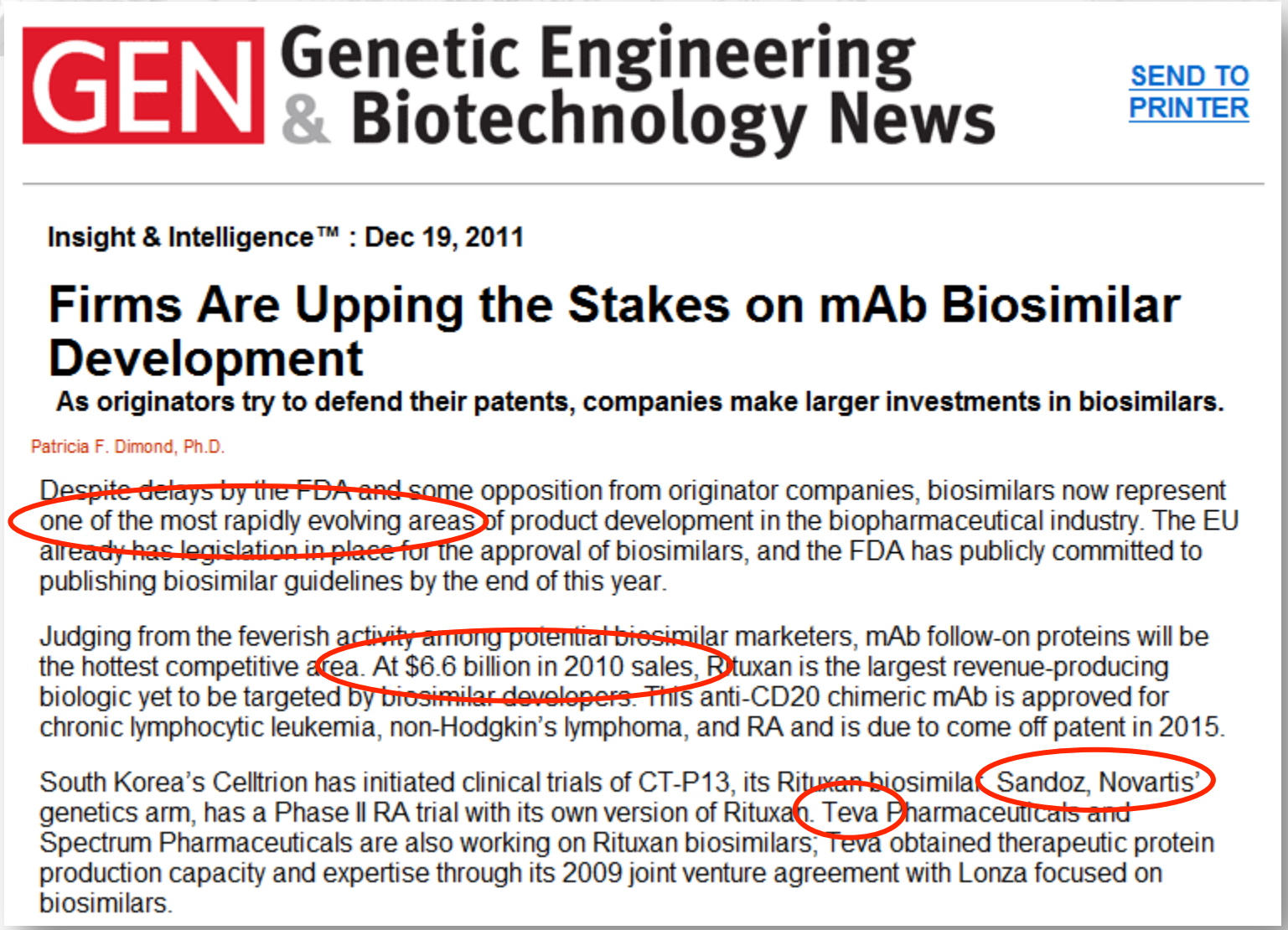
- Biosimilars
  - Need to demonstrate similarity/comparability between biosimilar to its innovator molecule
- Antibody Drug Conjugates (ADCs)
  - Increased analytical complexity due conjugate, linker & conjugation chemistries

BIOSIMILARS

Definitions

- Innovator biologic
  - Novel clinically-validated biologic on which biosimilars or biobetters are designed
- **Biosimilar**
  - **Biologic molecule with identical primary amino acid sequence as innovator biologic and developed with intention to be as close to the innovator product as possible**
- Biobetter
  - Biologic molecule based on the innovator molecule but with improvements intended to increase efficacy, potency, marketability, safety, or patient compliance
- Next-generation
  - Biologic molecule based on same validated target as innovator biologic, but with novel VH/VL chains and (typically) different epitope, with intent of making an improved biologic against the validated target

Biosimilars—*The Race is "ON"*



GEN Genetic Engineering & Biotechnology News

SEND TO PRINTER

Insight & Intelligence™ : Dec 19, 2011

Firms Are Upping the Stakes on mAb Biosimilar Development

As originators try to defend their patents, companies make larger investments in biosimilars.

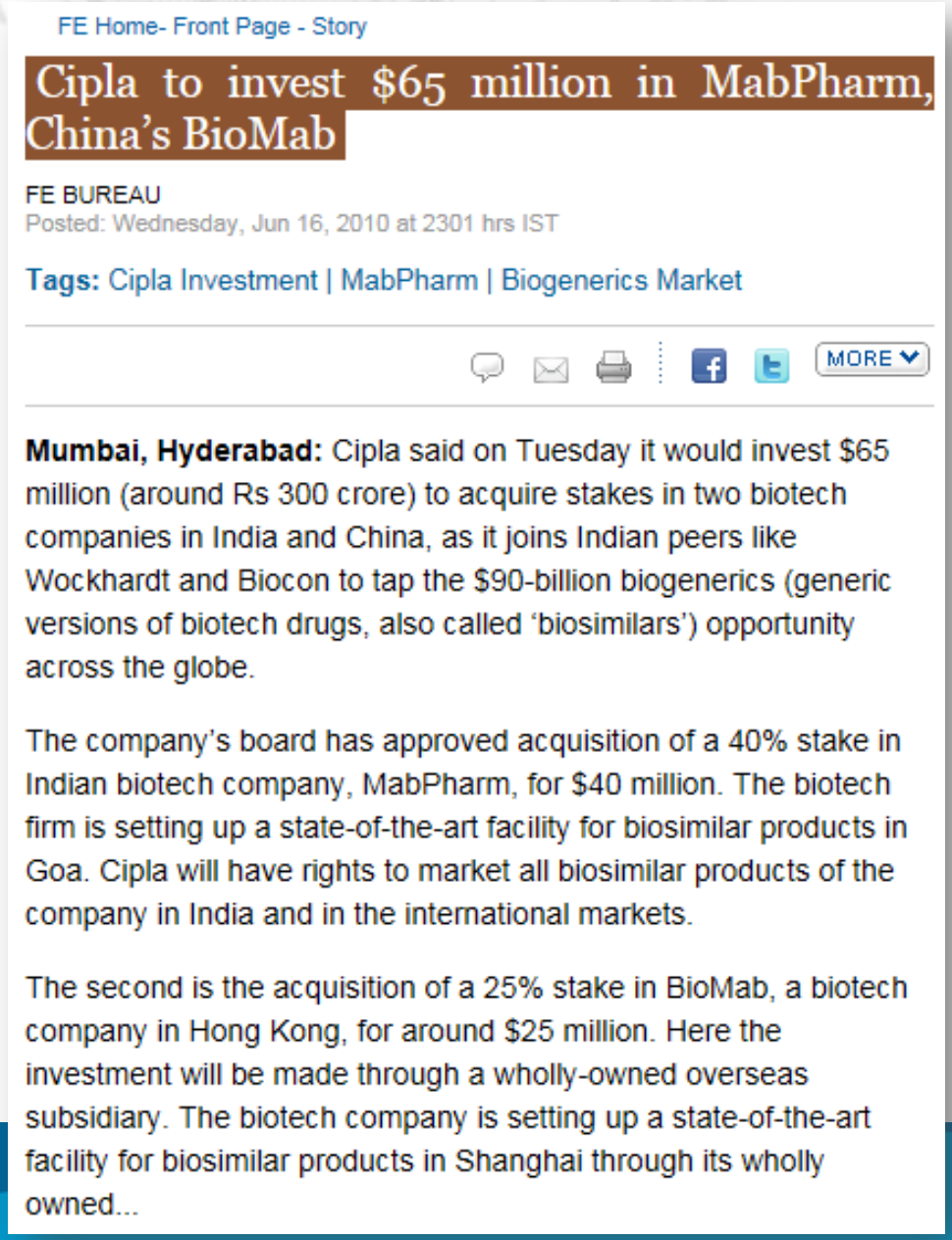
Patricia F. Dimond, Ph.D.

Despite delays by the FDA and some opposition from originator companies, biosimilars now represent one of the most rapidly evolving areas of product development in the biopharmaceutical industry. The EU already has legislation in place for the approval of biosimilars, and the FDA has publicly committed to publishing biosimilar guidelines by the end of this year.

Judging from the feverish activity among potential biosimilar marketers, mAb follow-on proteins will be the hottest competitive area. At $6.6 billion in 2010 sales, Rituxan is the largest revenue-producing biologic yet to be targeted by biosimilar developers. This anti-CD20 chimeric mAb is approved for chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and RA and is due to come off patent in 2015.

South Korea's Celltrion has initiated clinical trials of CT-P13, its Rituxan biosimilar. Sandoz, Novartis' genetics arm, has a Phase II RA trial with its own version of Rituxan. Teva Pharmaceuticals and Spectrum Pharmaceuticals are also working on Rituxan biosimilars; Teva obtained therapeutic protein production capacity and expertise through its 2009 joint venture agreement with Lonza focused on biosimilars.

Biosimilars—*"Foot-in-the-Door"* for Emerging Markets



FE Home- Front Page - Story

Cipla to invest $65 million in MabPharm, China's BioMab

FE BUREAU
Posted: Wednesday, Jun 16, 2010 at 2301 hrs IST

Tags: Cipla Investment | MabPharm | Biogenerics Market

MORE

Mumbai, Hyderabad: Cipla said on Tuesday it would invest $65 million (around Rs 300 crore) to acquire stakes in two biotech companies in India and China, as it joins Indian peers like Wockhardt and Biocon to tap the $90-billion biogenerics (generic versions of biotech drugs, also called 'biosimilars') opportunity across the globe.

The company's board has approved acquisition of a 40% stake in Indian biotech company, MabPharm, for $40 million. The biotech firm is setting up a state-of-the-art facility for biosimilar products in Goa. Cipla will have rights to market all biosimilar products of the company in India and in the international markets.

The second is the acquisition of a 25% stake in BioMab, a biotech company in Hong Kong, for around $25 million. Here the investment will be made through a wholly-owned overseas subsidiary. The biotech company is setting up a state-of-the-art facility for biosimilar products in Shanghai through its wholly owned...

…and large corporations like Samsung

GEN News Highlights: Dec 6, 2011

Biogen Idec, Samsung Establish $300M Biosimilar Joint Venture

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KEYWORDS: Asia, Biogen Idec, Bi
Biologics

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(Page 1 of 1)

Biogen Idec and Samsung establishe
based in Korea, will be led by Samsur
contract with Biogen Idec and Samsu
state. However, it won't look to develo

Samsung Biologics was established in
will allow us to leverage our world-clas
focus on our mission of discovering, d
neurodegenerative diseases, hemophi
CEO. "We are very impressed with Samsung's track record of leadership and excellence in all their businesses."

Today's Top Stories

1. Samsung will make some Roche biologics

By Eric Palmer

Comment | Forward | Twitter | Facebook | LinkedIn

Roche ($RHHBY), the leader in biologic drugs, this month laid out a blueprint for a 5-year buildout of its biologics manufacturing network. But even after nearly $900 million in investments and the addition of 500 jobs, the Swiss drugmaker will turn for some products to Samsung BioLogics, a company that has worked on a knockoff of at least one of Roche's drugs.

The South Korean company, which has pledged $2 billion in investments to become an expert in biologics manufacturing, said Wednesday it and Roche had clinched a "long-term strategic manufacturing agreement." Samsung will manufacture an undisclosed number of Roche's cell-based products at its two manufacturing facilities in Incheon, South Korea.

Amgen’s biosimilars push

Tuesday, February 19, 2013

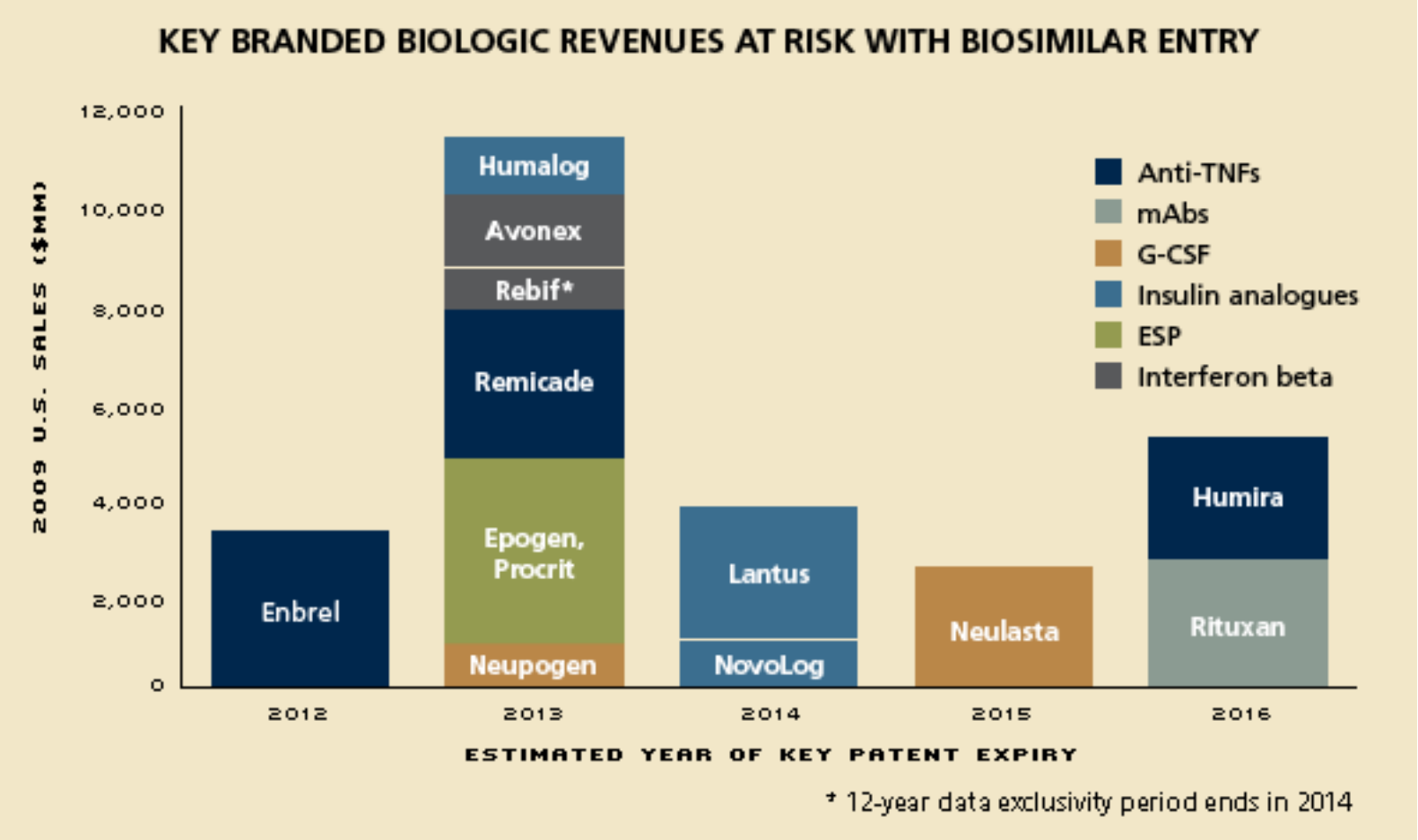
Amgen's Biosimilars Gambit

About a week ago, Amgen rocked the biotech industry's proverbial boat with their announcement that they'd be entering the biosimilars market. Multiple news outlets like Yahoo!, Forbes, and CNBC report that Amgen, starting in 2017, will be making six generic versions of blockbuster biologics:

- Abbvie's Humira
- Janssen's[OLY 02/19/13] Remicade
- Roche's Avastin, Herceptin and Rituxan
- Eli Lilly's Erbitux

This comes as a surprise to many, because for years, Amgen has been saying that biologics really can't be copied.

Biologics are falling off the patent cliff too!



BPCI* Act defines Biosimilar or Biosimilarity

- Biosimilar or Biosimilarity means:
  - that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components; and
  - there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product

- FDA Biosimilars Guidance Outlines 'Stepwise' Development Approach
  - The FDA has issued three long-awaited biosimilars guidance documents, recommending a stepwise approach to showing biosimilarity that could allow eased trial requirements if a sponsor can demonstrate biosimilarity in earlier steps

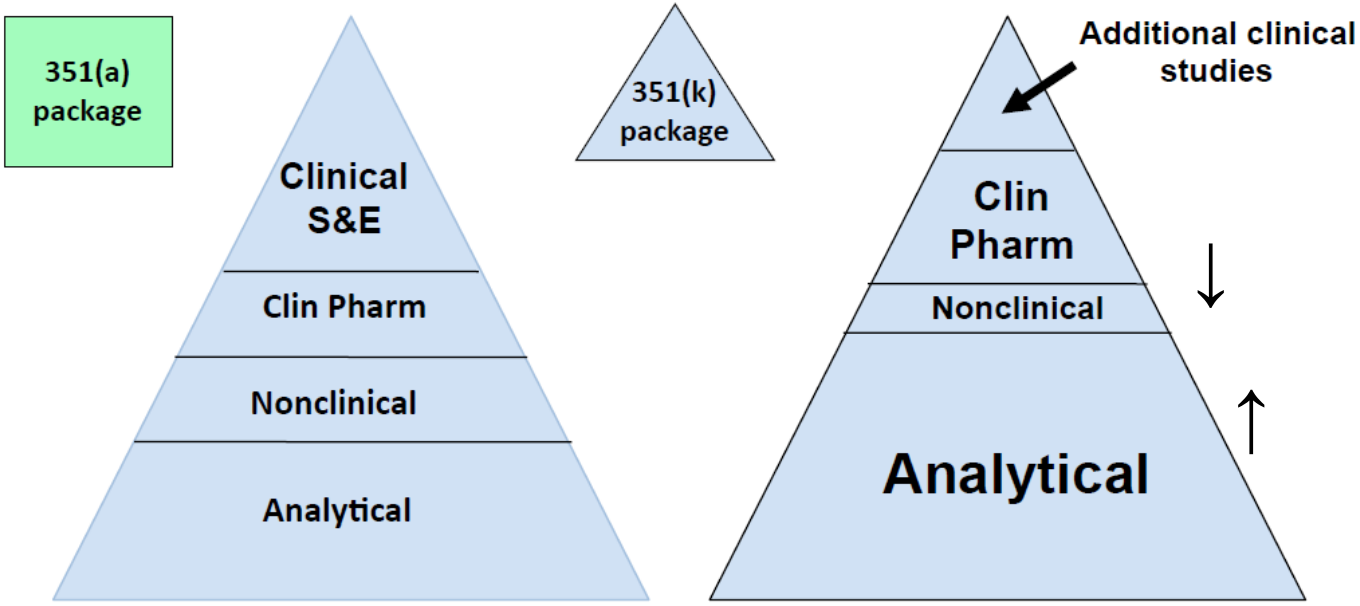
*Biologics Price Competition and Innovation Act of 2009

The challenge
How similar is similar enough?

- Which attributes matter and which don't?
- If differences are seen, they need to be shown, somehow, not to be clinically meaningful
- To **predict clinical similarity from the analytical data**, need to understand relationship between protein quality attributes and the clinical safety & efficacy profile of the specific product
- What does the demonstration of "highly similar" get you?
  - An Abbreviated Licensure Pathway
    - Licensure based on less than the full complement of product-specific non-clinical and clinical data that would normally be required for a new biological entity

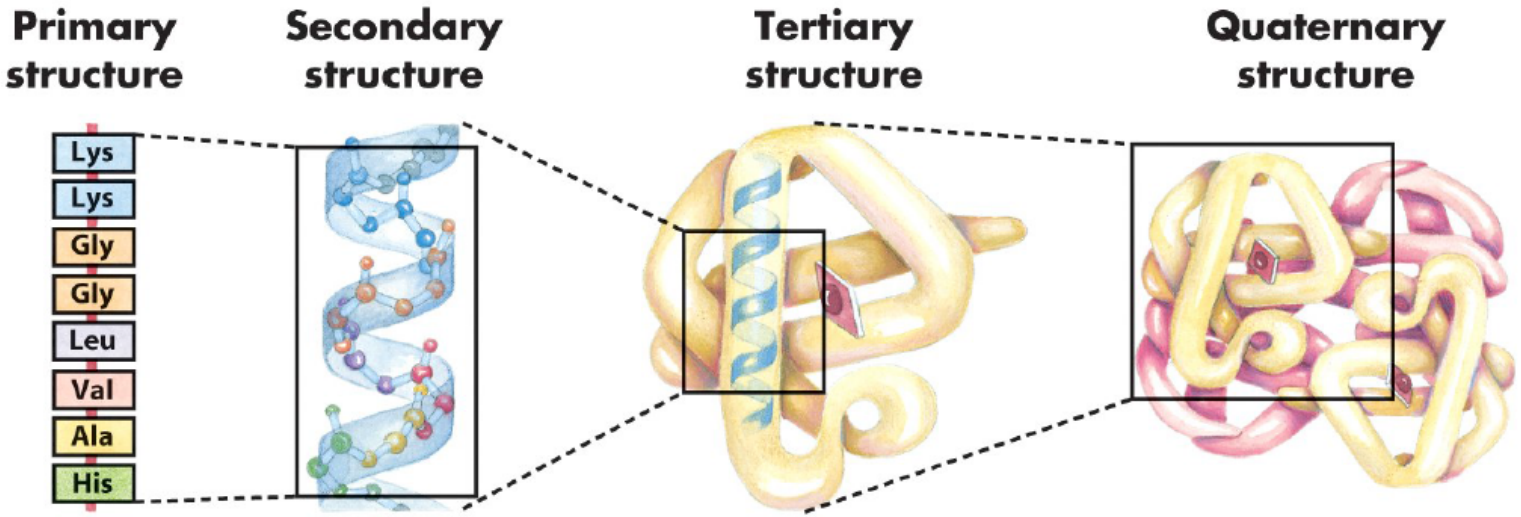
New paradigm for biosimilar development
Scientific Considerations Draft Guidance

- The stepwise approach should start with extensive structural and functional characterization of both the proposed product and the reference product, which serves as the foundation of a biosimilar development program



- Highly similar analytical & PK/PD data = ↓ Risk of clinical differences
  - Reduce requirements for clinical studies

What does extensive structural and functional characterization means?



Amino acid sequence
Charge
hydrophobicity

Helix, β sheet, turns & loops

Folded polypeptide chain, disulfide bonds, forces

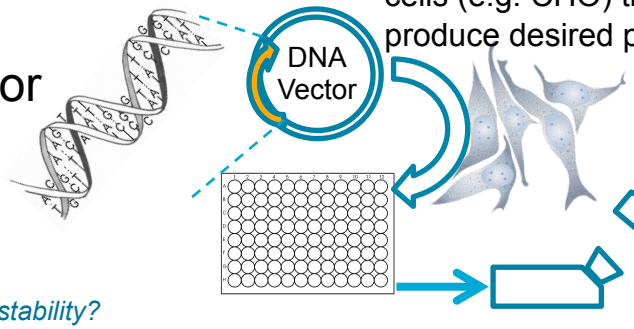
Subunits, protein-protein interactions, aggregates, higher order structure

- All need to be evaluated as part of analytical similarity studies

Different process, different product

Biosimilar that is different from the originator

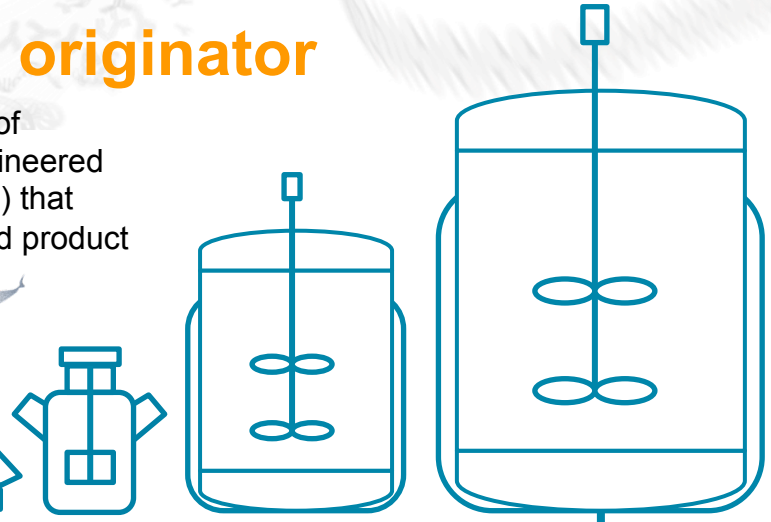
Same gene as innovator



Establishment of genetically engineered cells (e.g. CHO) that produce desired product

Same amino acid seq as innovator?
N- or C-terminal truncation?
Any modification because of genetic stability?

Did host cell protein impurity profile change?
Glycosylation profile?



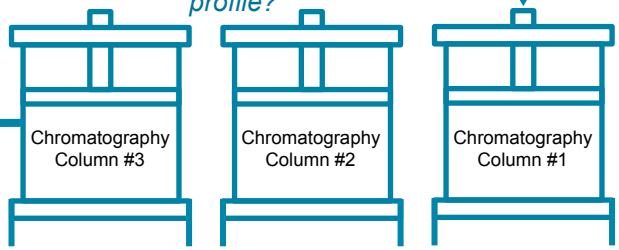
Cell Culture Scale-up & Production

Process- and product-related substances and impurities?
Raw material analysis?

Centrifuge

Even if a biosimilar uses the same human gene as its innovator, it will differ in other parts of manufacturing process

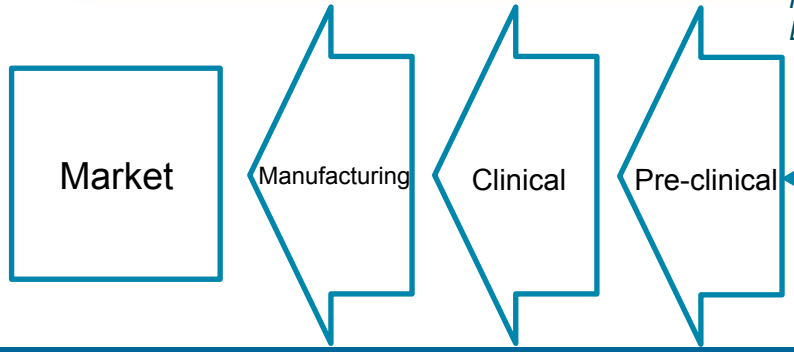
Stability studies
Product degradation profile?



Downstream Processing & Purification

Formulation & excipients
Effect on stability, safety & efficacy?

Formulation

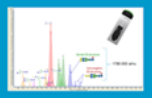


Analytical tools to evaluate biosimilarity are the same but focus on comparability features

Attributes	Analytical tools
Amino acid sequence and modifications	Mass spectrometry (MS), peptide mapping, chromatographic separations
Folding	S-S bonding, calorimetry, HDX and IM-MS, NMR, circular dichroism, Fourier transform & Raman spectroscopy, fluorescence, interaction chromatographies
Subunit interactions	Chromatography, IM-MS
Heterogeneity (size, charge, hydrophobicity)	Chromatography resins; gel & capillary electrophoresis, light Scattering, IM-MS
Glycosylation	Anion exchange, enzymatic digestion, peptide mapping, CE, MS
PEGylation & isomers	Chromatography, peptide mapping
Bioactivity	Cellular and animal bioassays; ligand & receptor binding (ELISA, surface plasmon resonance), signal transduction
Aggregation	Analytical ultracentrifugation, size-exclusion chromatography, field flow fractionation, light scatter, microscopy
Proteolysis	Electrophoresis, chromatography, MS
Impurities (HCP, DNA)	LC, LC/MS, LBAs, PCR, metal (ICP-MS) & solvent analysis

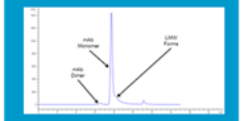
We have the most comprehensive portfolio of analytical instrumentation & solutions for biosimilars

Glycan Analysis



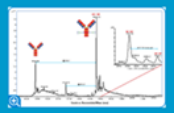
CE-LIF, CE-MS
LC-FLD, LC-MS
LC-Chip/MS mAb-Glyco kit

Aggregation



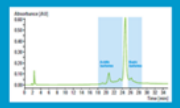
LC-UV Size Exclusion
CE-UV
Field-flow fractionation

Molecular Weight Determination

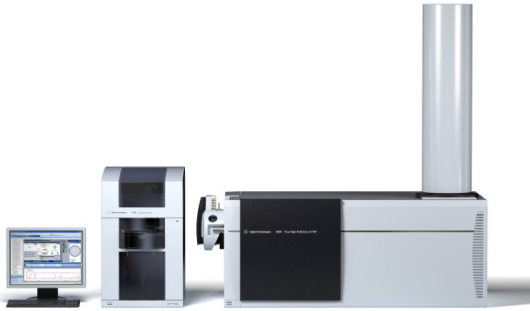


CE SDS-PAGE, CE-MS
Microfluidic SDS-PAGE
LC-UV or LC-MS

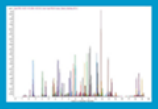
Charge Variants



IEF analyzers (iCE 280)
CE (cIEF), CE-MS
Bio-LC-UV Ion Exchange

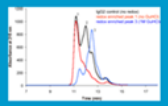


Peptide Characterization/ Mapping



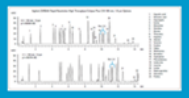
LC-UV, LC-MS
CE-UV, CE-MS

Oxidation



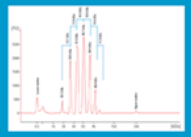
CE-MS
LC-UV , LC-MS
HIC and Reversed Phase

Amino Acids



LC-UV , LC-MS
CE, CE-MS

Protein PEGylation



LC-UV SEC / Cation Exchange
Microfluidic SDS-PAGE

Comparison of follow on biologics to an innovator mAb by HPLC, SEC and Peptide Mapping

Samples:

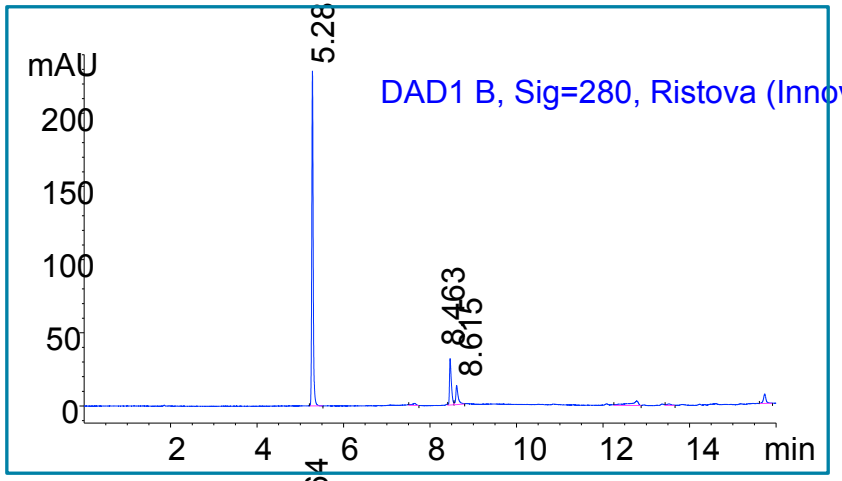
- Innovator – **Ristova** (Rituximab/Roche)
- Biosimilar – **Reditux** (Rituximab/Dr. Reddy's)
  - Samples purchased from local Pharmacy in Bangalore, India

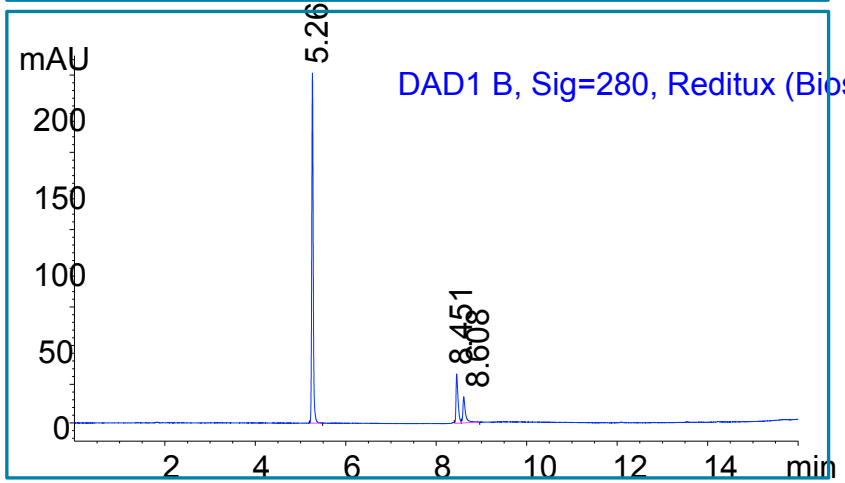
Analytical tools:

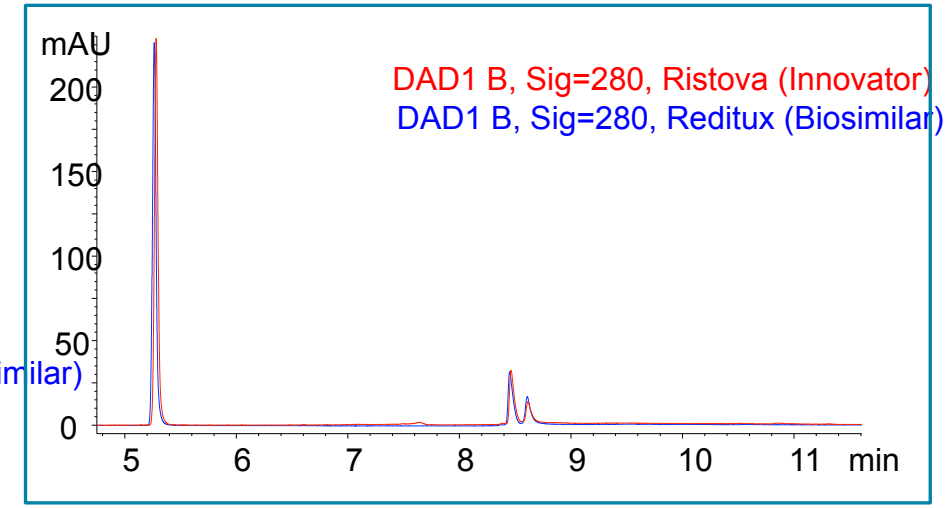
- The Agilent 1260 Bio-inert LC
- Biocolumns
- Match Compare Software

RP HPLC of Biosimilar and Innovator mAb

Agilent 1260 Infinity Bio-inert LC using Poroshell 120 SB C18 4.6x150 mm, 2.7 µm column



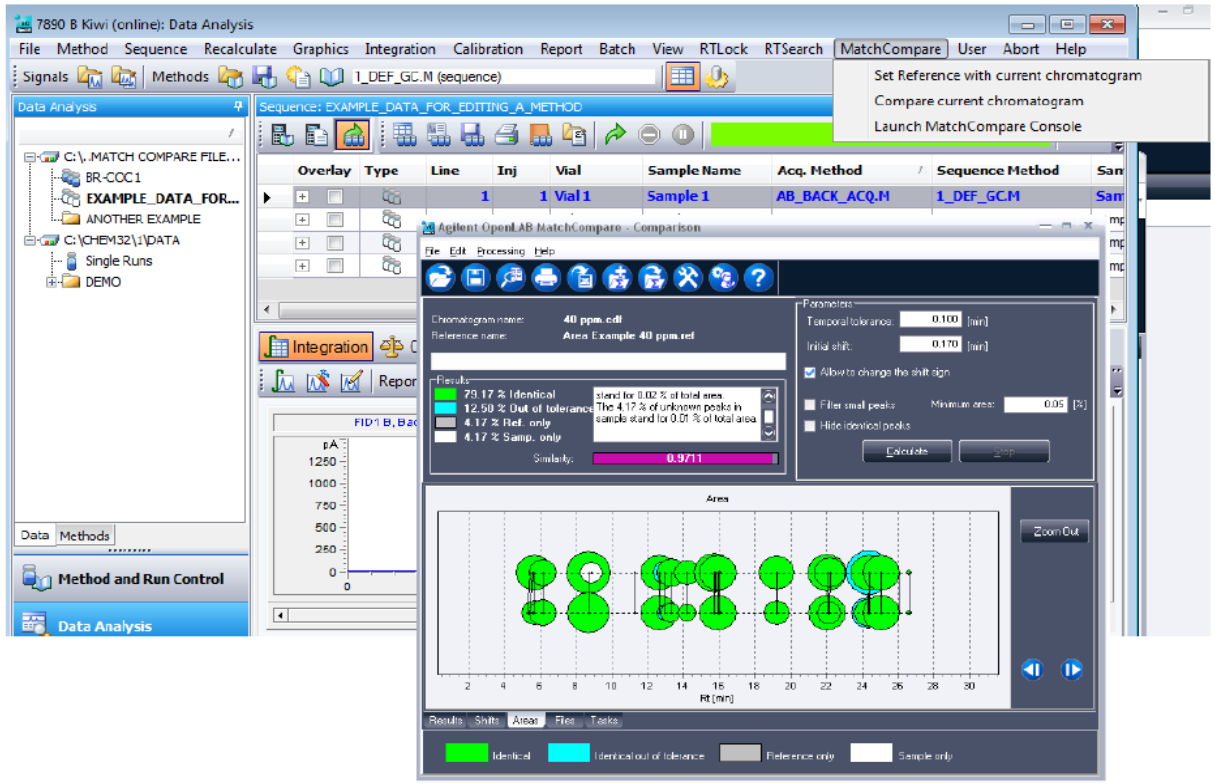




Agilent Match Compare tool for comparison

Compare an unknown sample, by selecting the sample chromatogram, in data analysis within OpenLAB CDS.

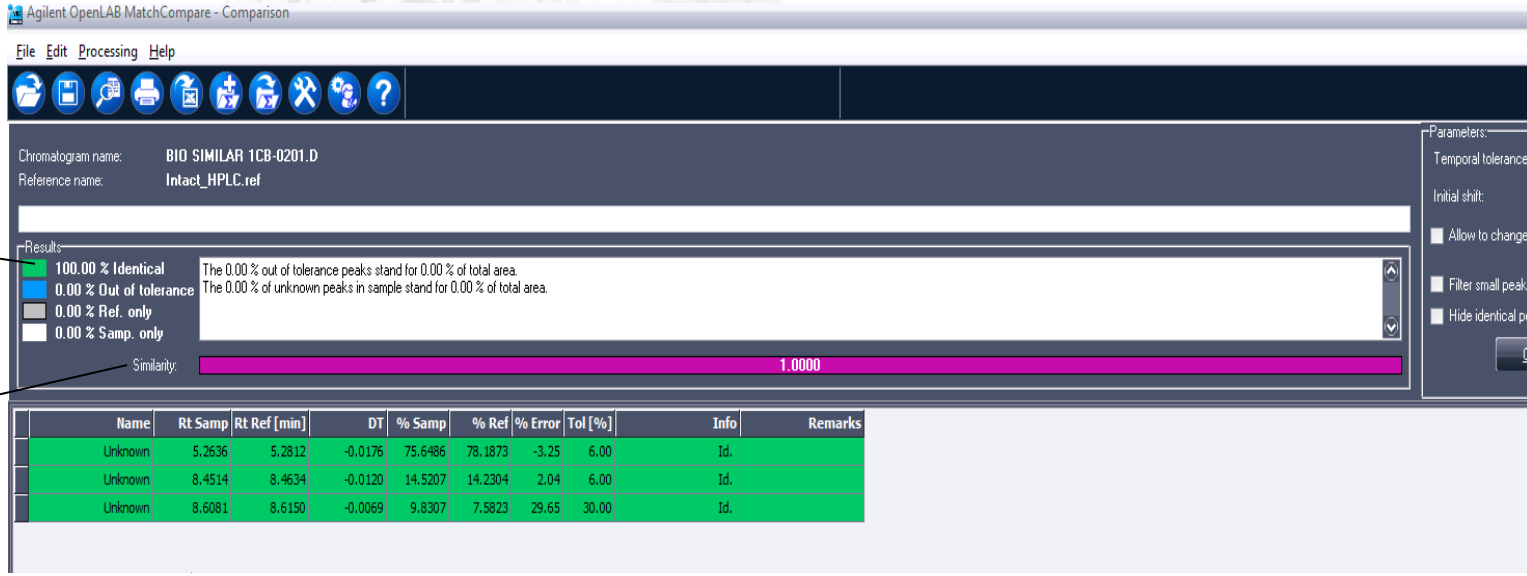
To start the comparison, select “Compare current chromatogram” under the Match Compare menu item.

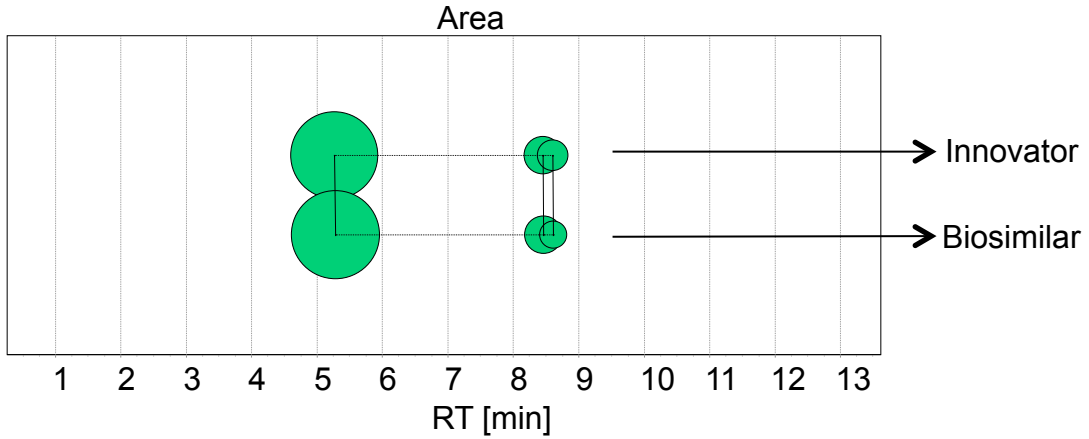


Agilent Match compare analysis of intact mAbs – RP HPLC

100% identical

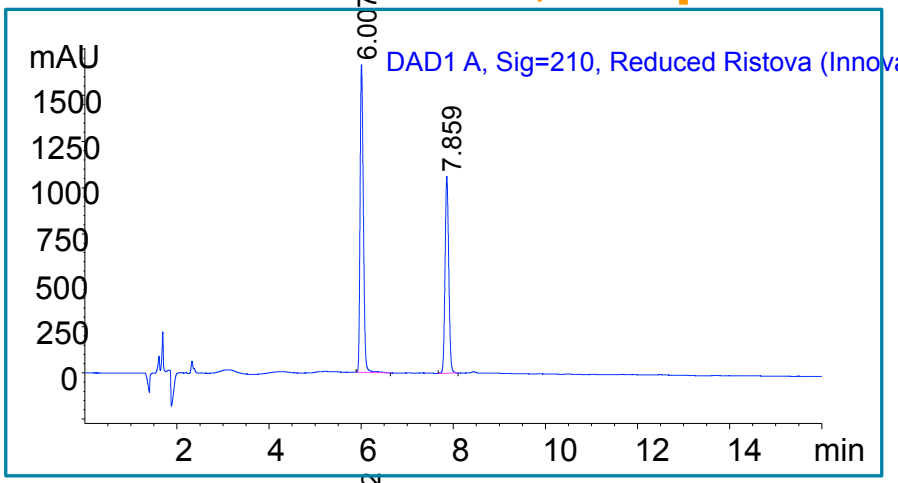
Similarity 1.000

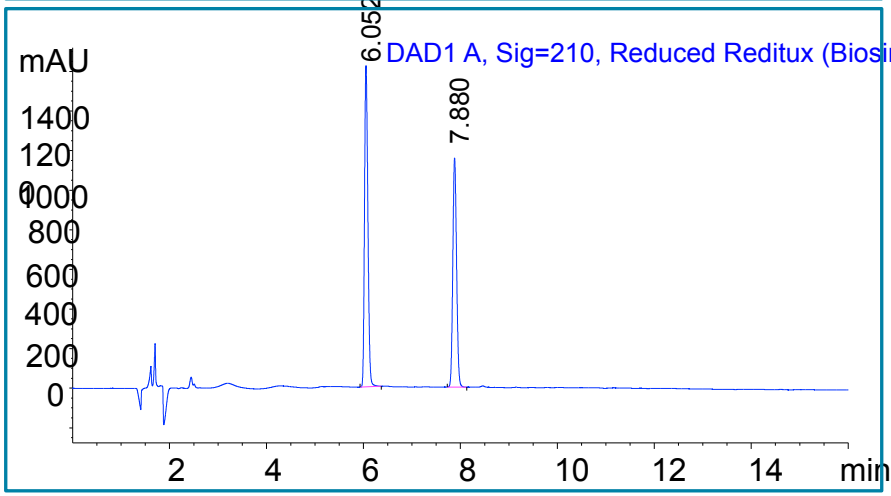


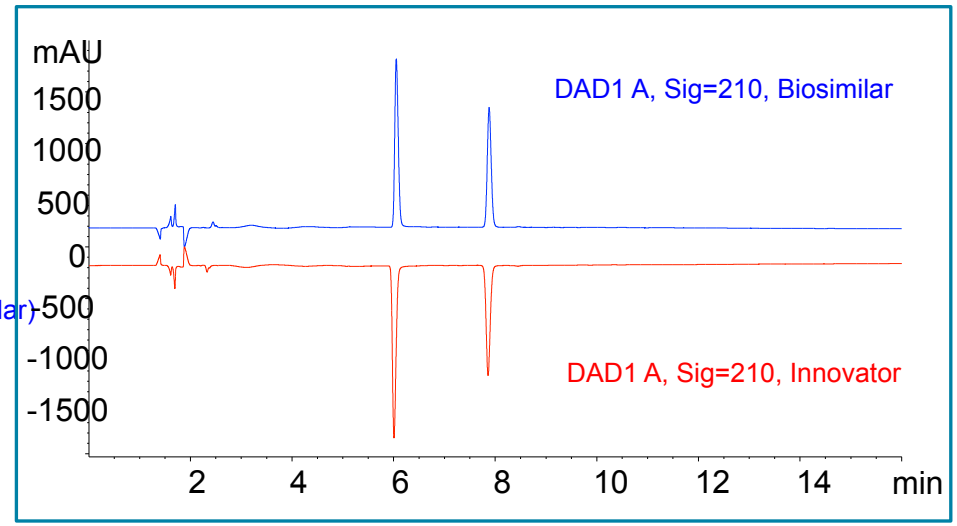


RP HPLC of reduced biosimilar and innovator mAb

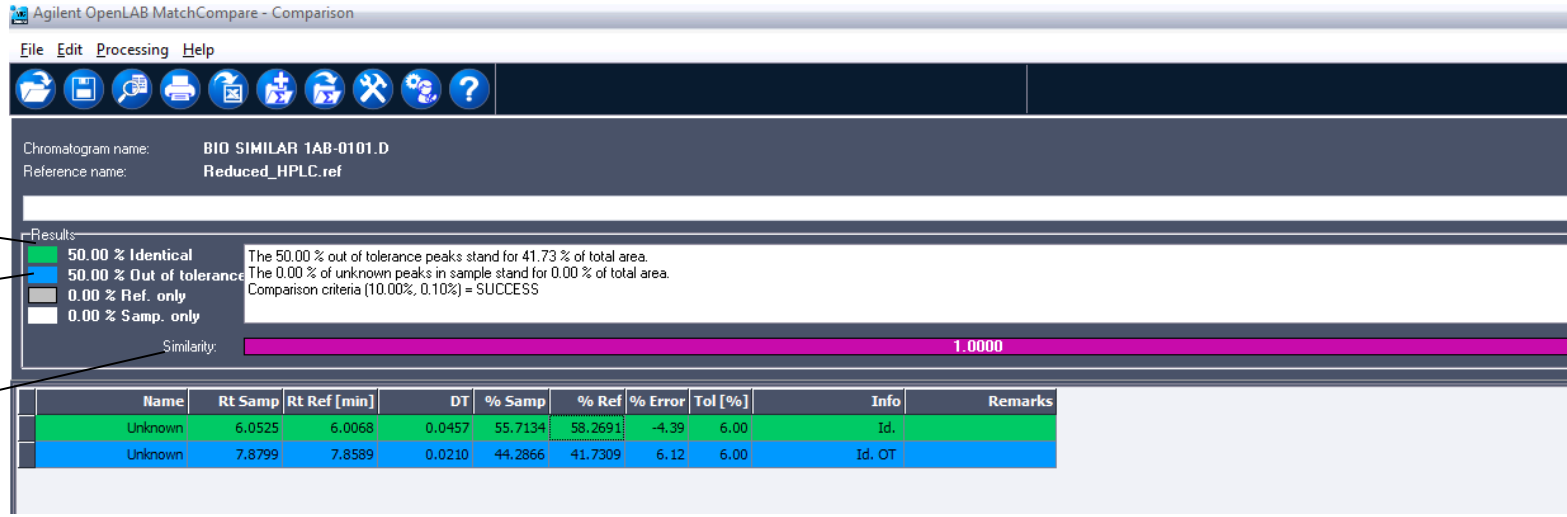
Agilent 1260 Infinity Bio-inert LC using Poroshell 120 SB C18 4.6x150 mm, 2.7 µm column







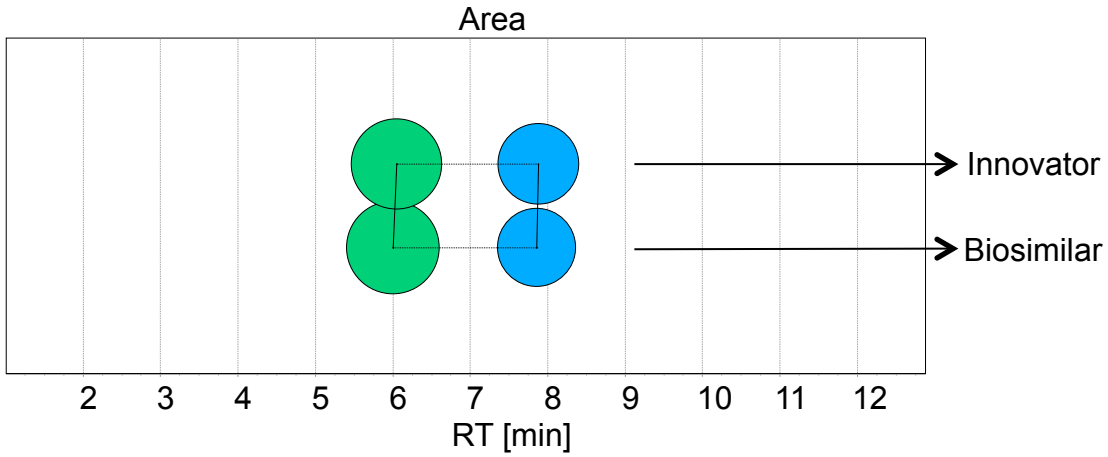
Agilent Match compare analysis of reduced mAb-HPLC



50% identical

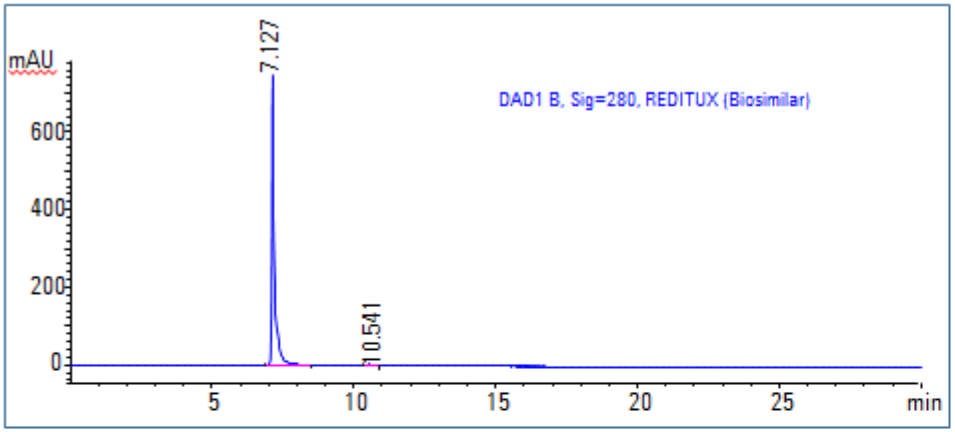
50% out of tolerance

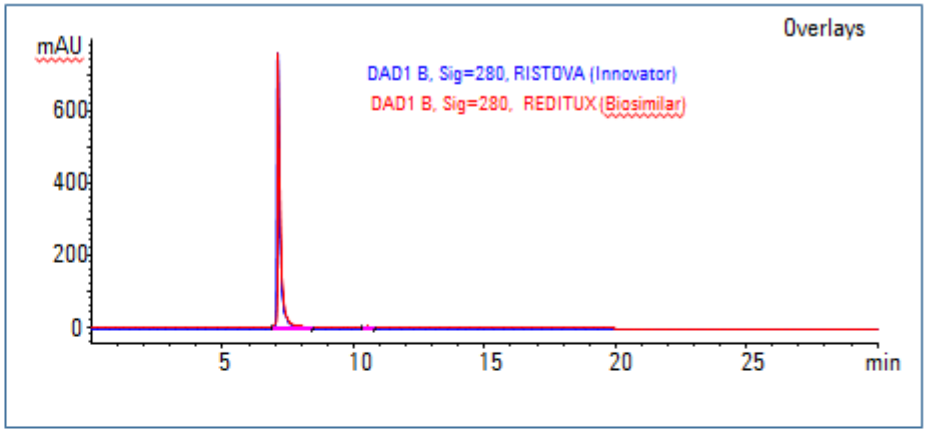
Similarity 1.000

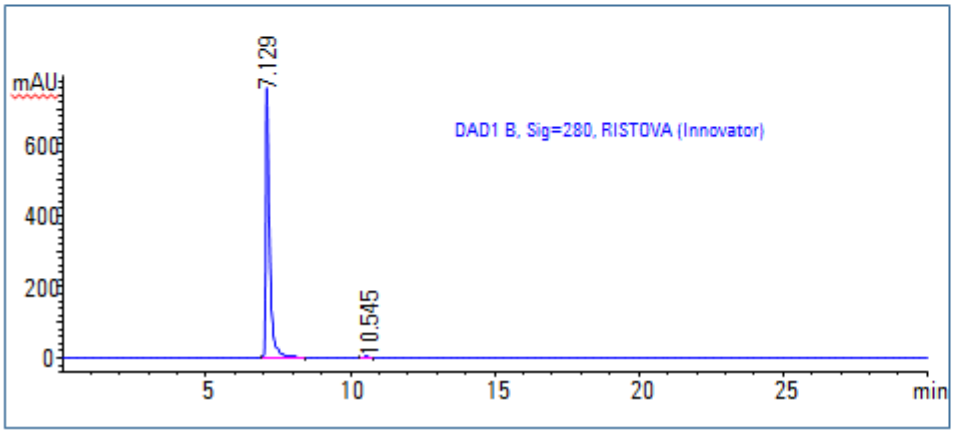


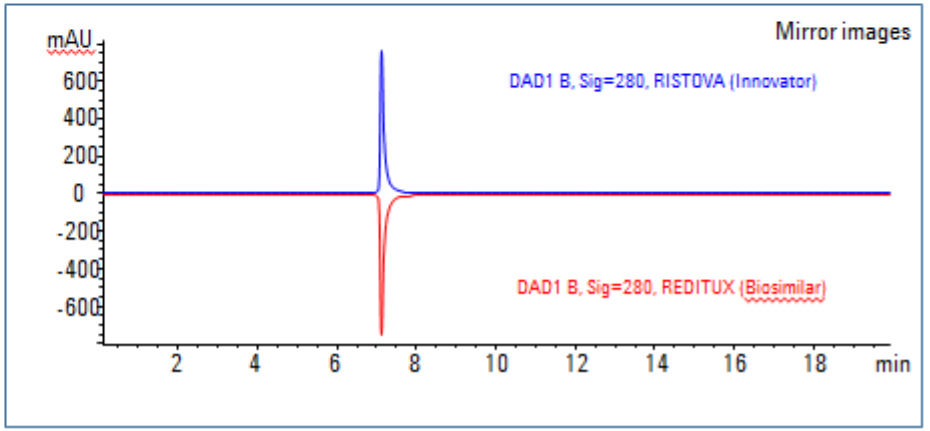
Intact SEC of Biosimilar and Innovator mAb

Agilent 1260 Infinity Bio-LC using a Bio SEC-3, 300Å, 7.8x300 mm, 3 µm









Agilent Match compare analysis of intact mAbs-SEC

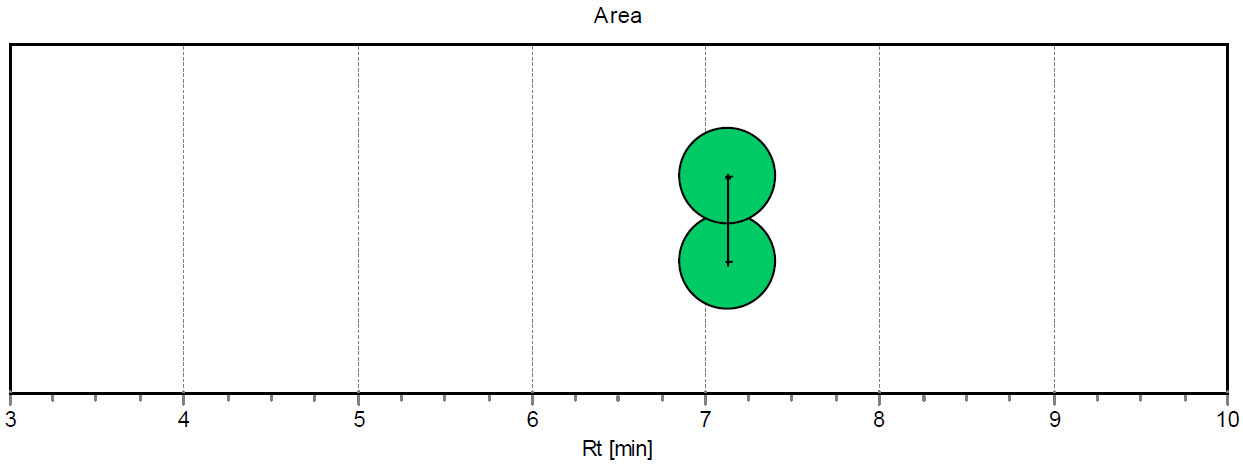
Results table

Code	Name	Rt Samp [min]	Rt Ref [min]	DT	% Samp	% Ref	% Error	Tol [%]	Info
ID	Unknown	7.1308	7.1292	0.0016	100.0000	100.0000	0.00	6.00	Id.

0.00 % of peaks out of tolerance represents 0.00 % of total area

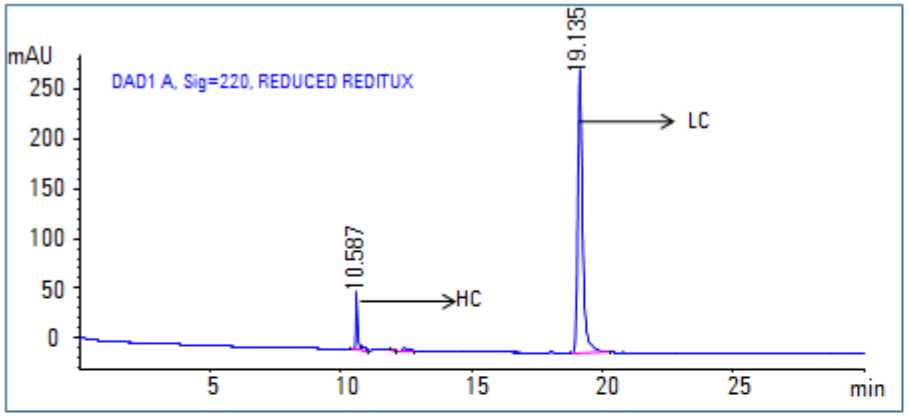
0.00 % of unknown peaks represents 0.00 % of total area

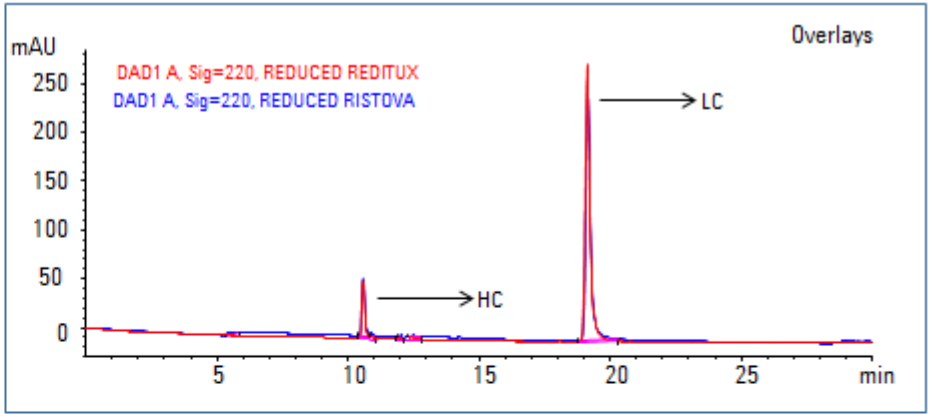
Area

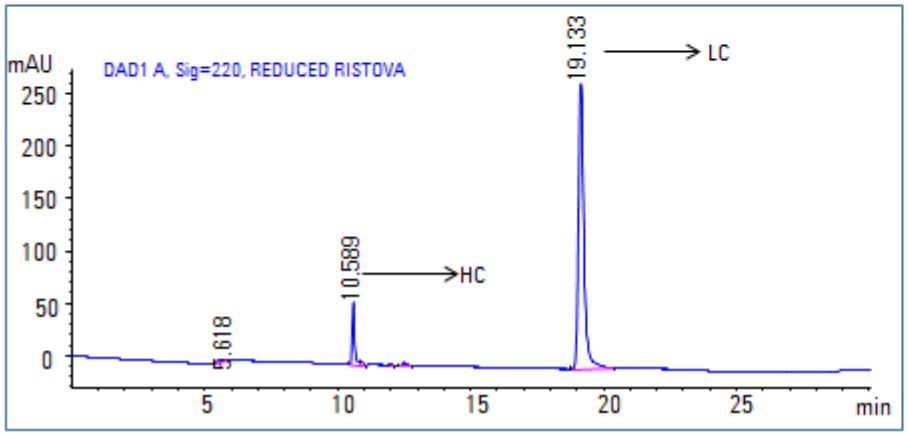


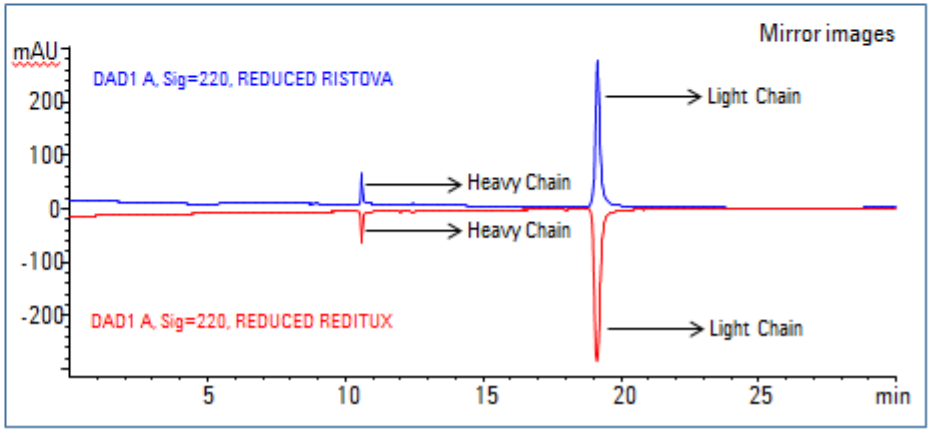
SEC of Reduced Biosimilar and Innovator mAb

Agilent 1260 Infinity Bio-LC using a Bio SEC-3, 300Å, 7.8x300 mm, 3 µm

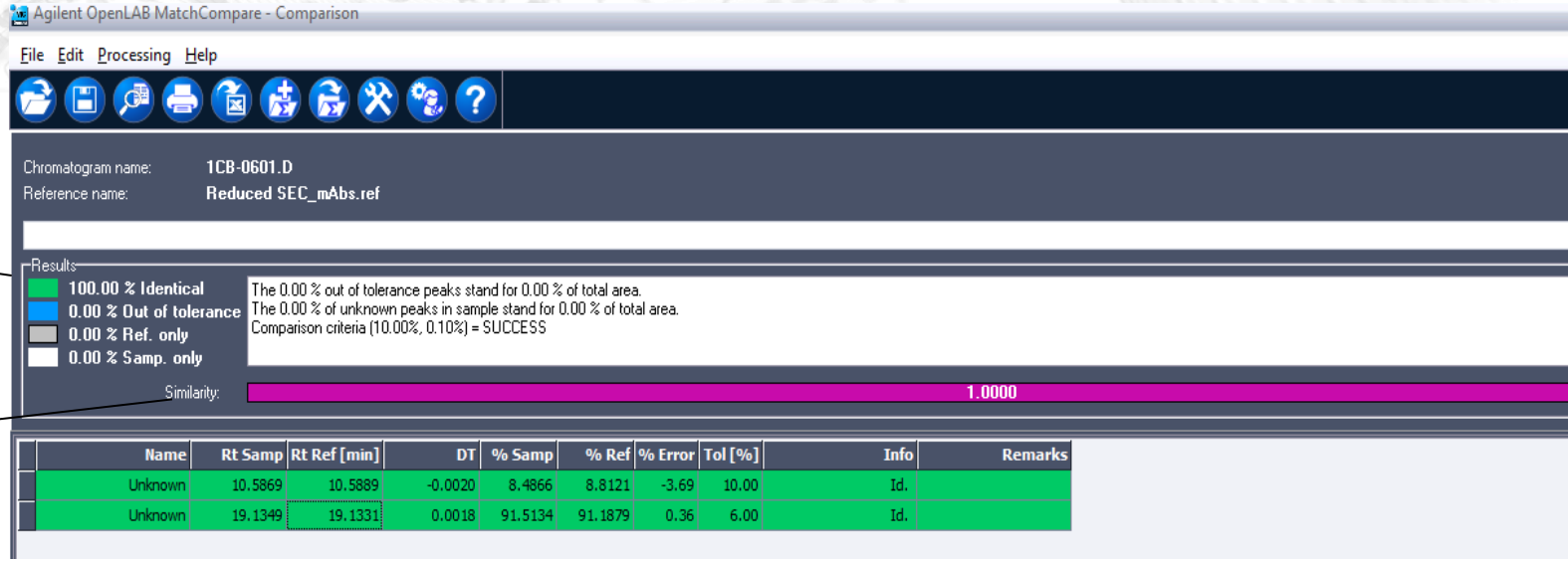






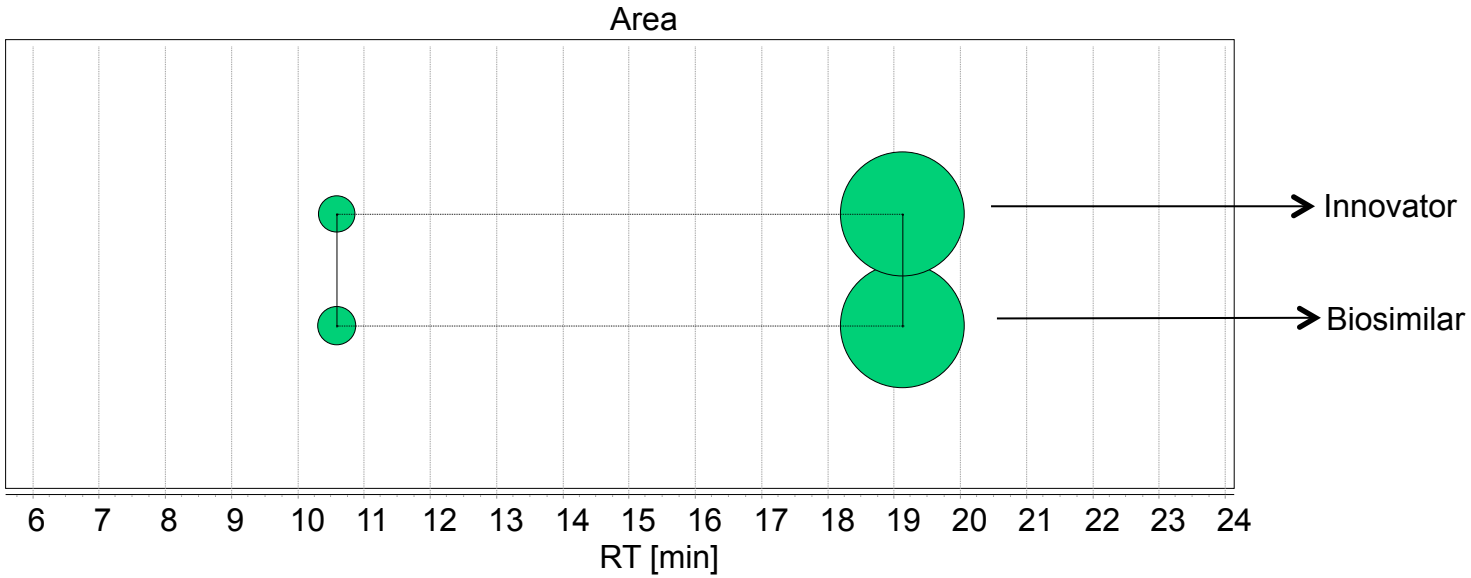


Match compare analysis of reduced mAbs – SEC



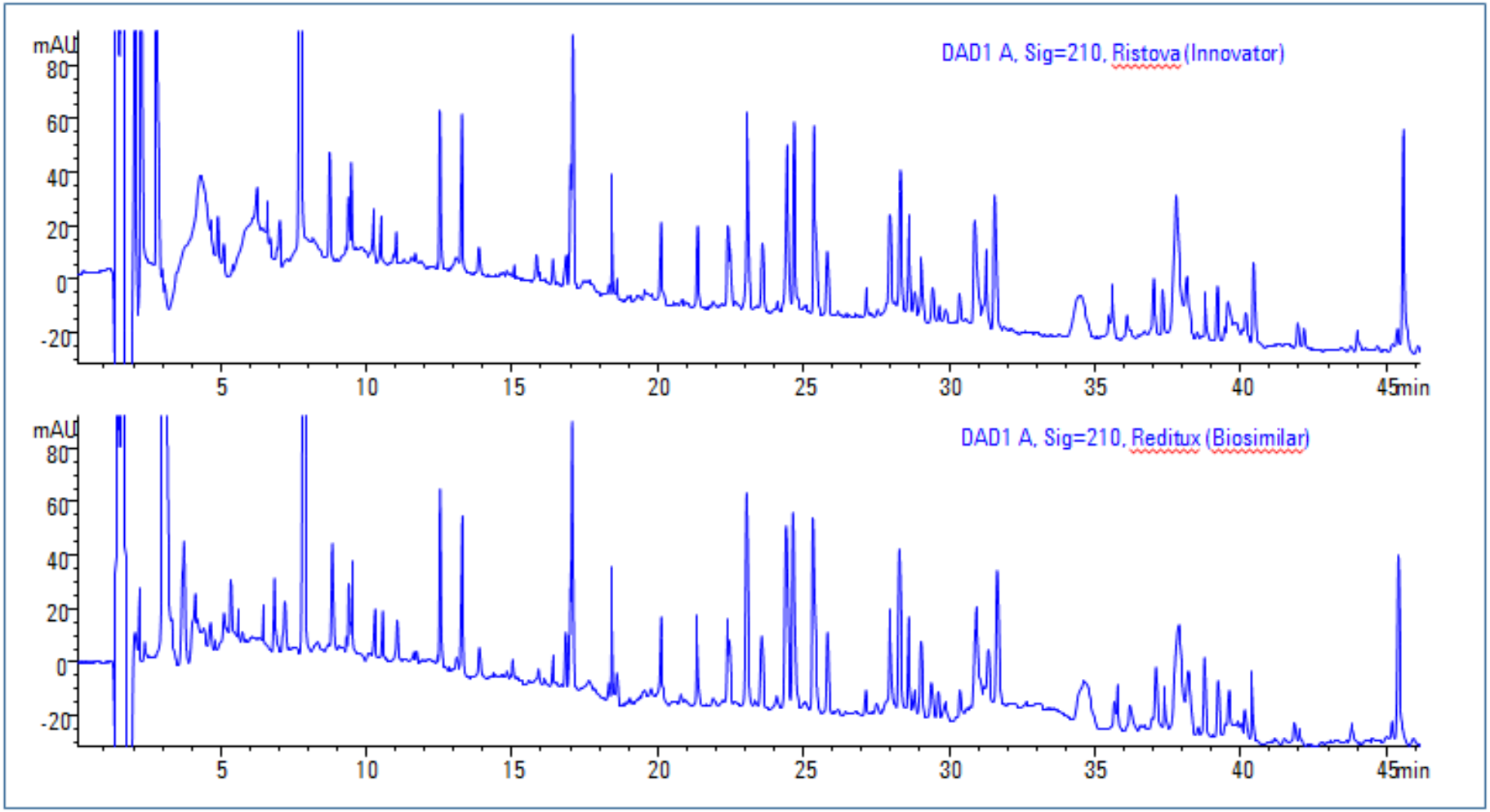
100% identical

Similarity 1.000



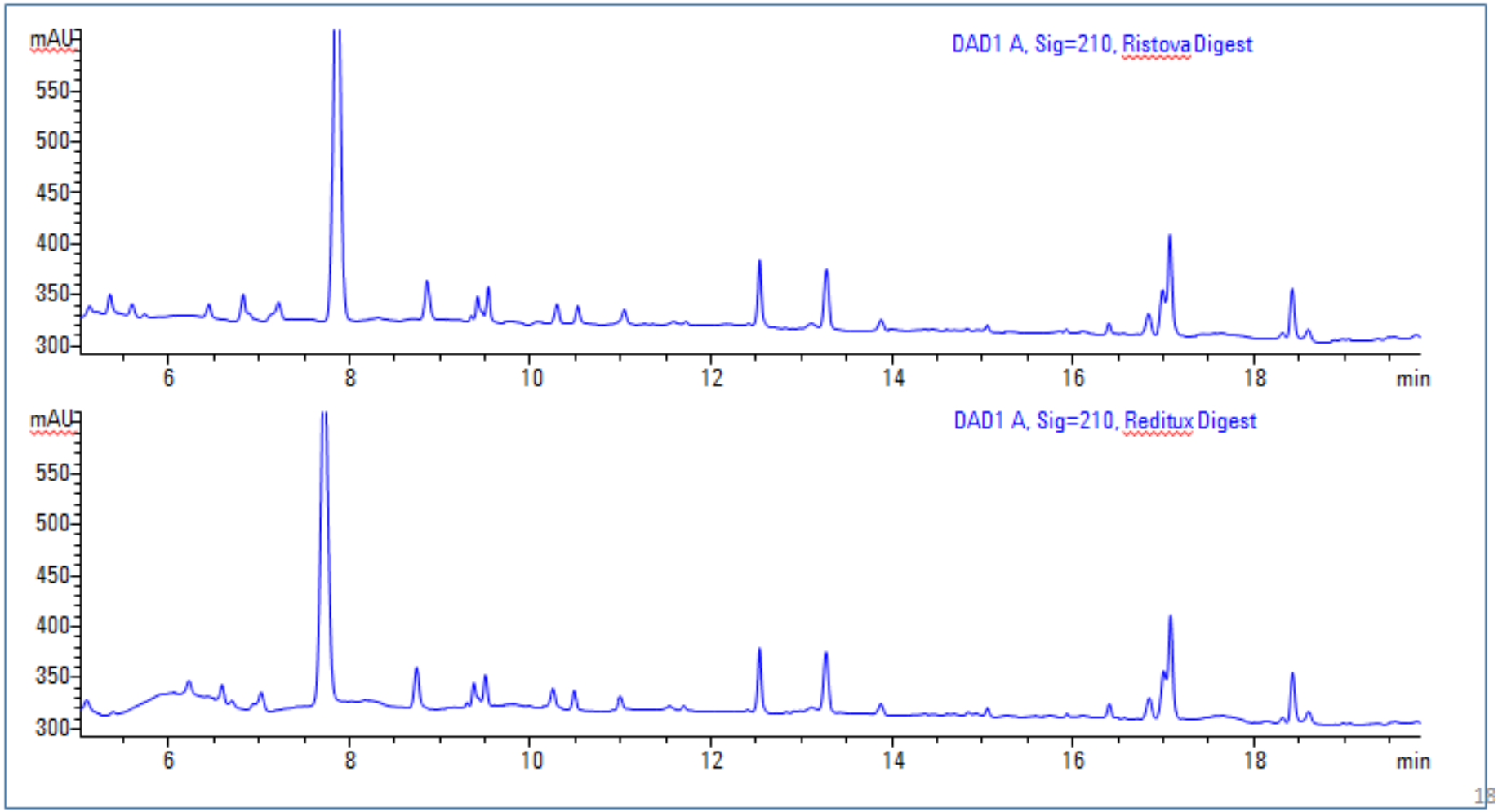
Peptide mapping of biosimilar and innovator mAb

Agilent 1260 Infinity Bio-LC using a Poroshell 120 SB C18 4.6x150 mm. 2.7 um column



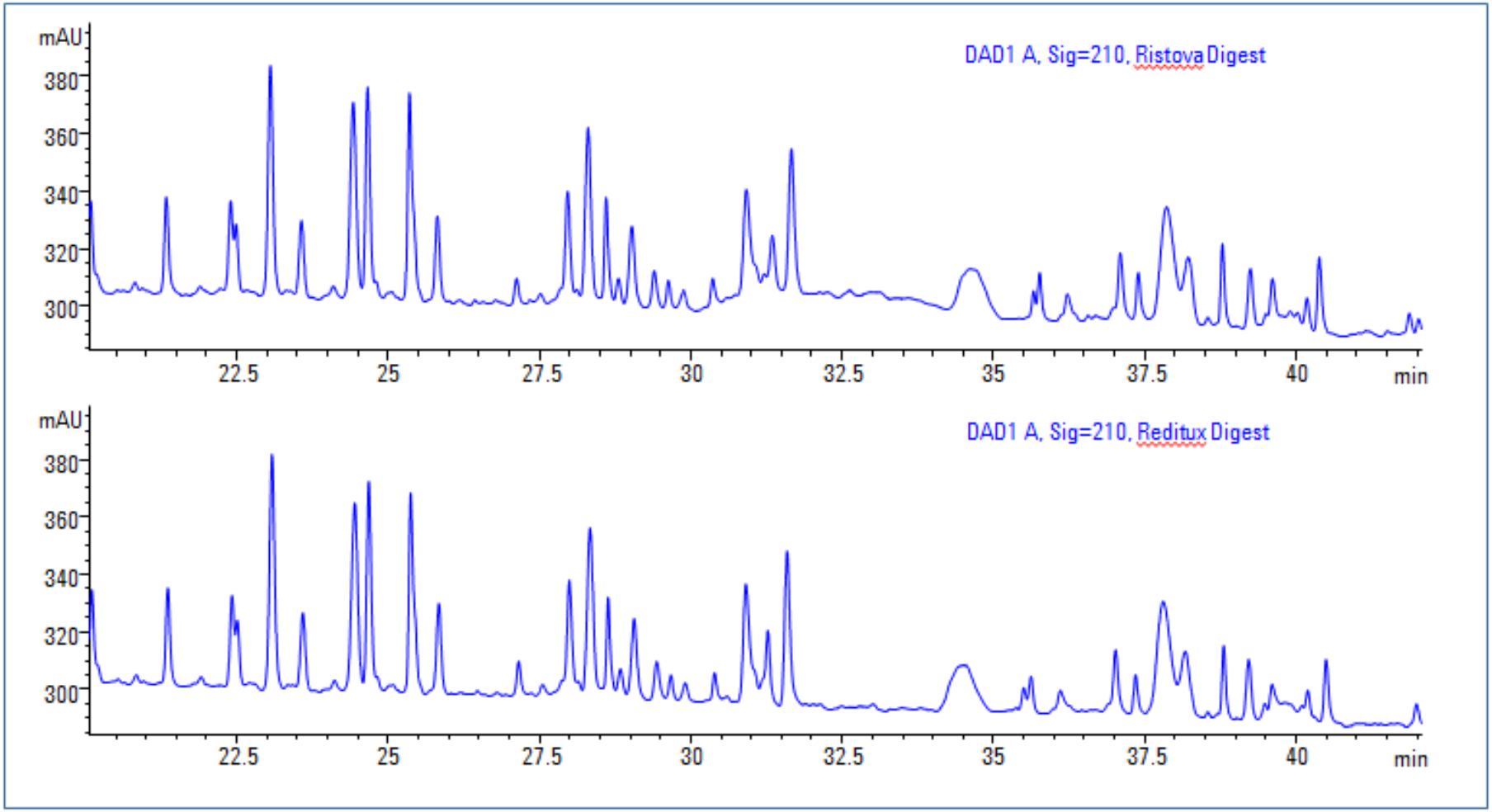
Peptide mapping of Biosimilar and innovator mAb

Zoom in of chromatogram; 5 – 20 min

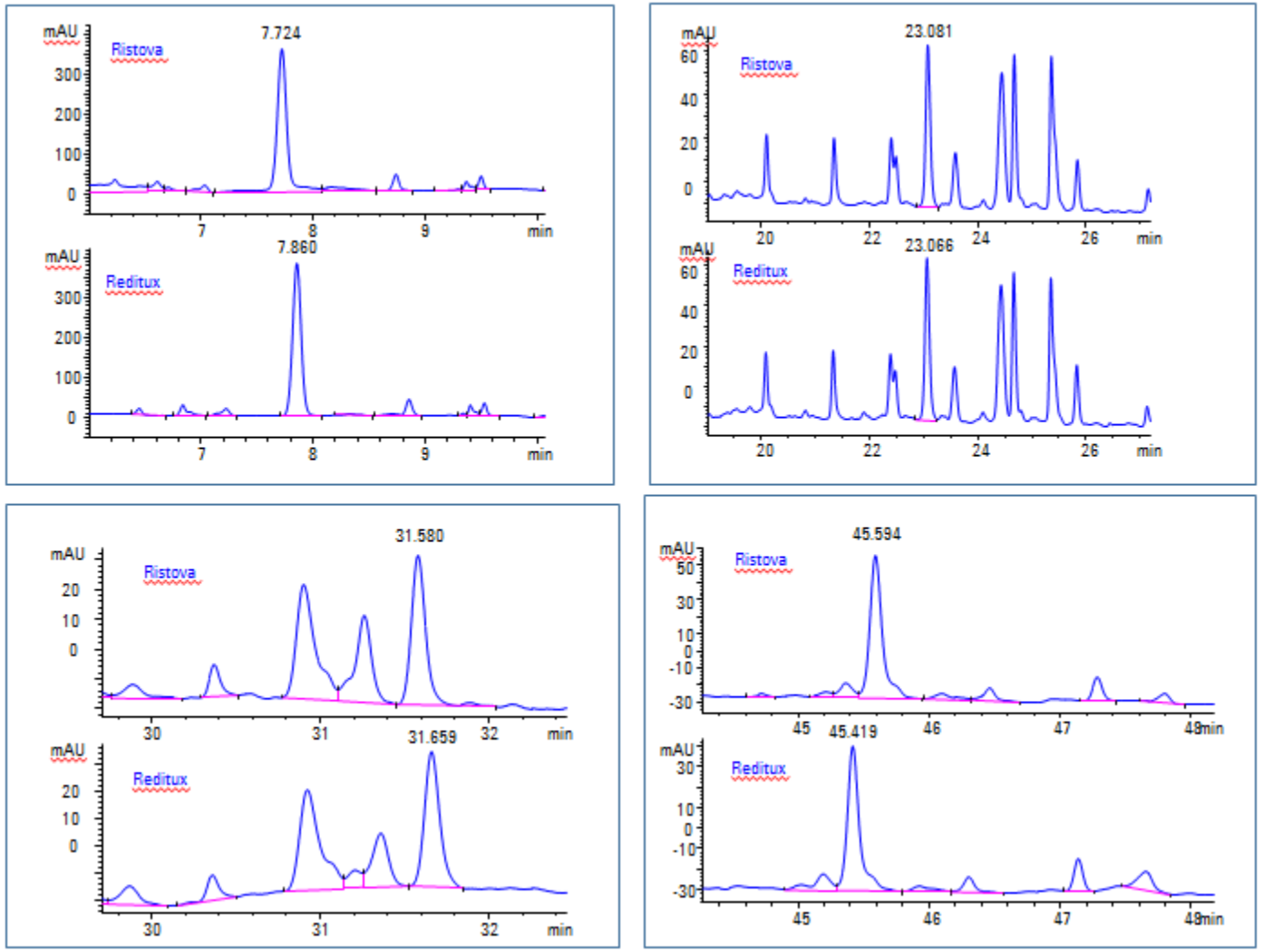


Peptide mapping of Biosimilar and innovator mAb

Zoom in of chromatogram; 20 – 40 min

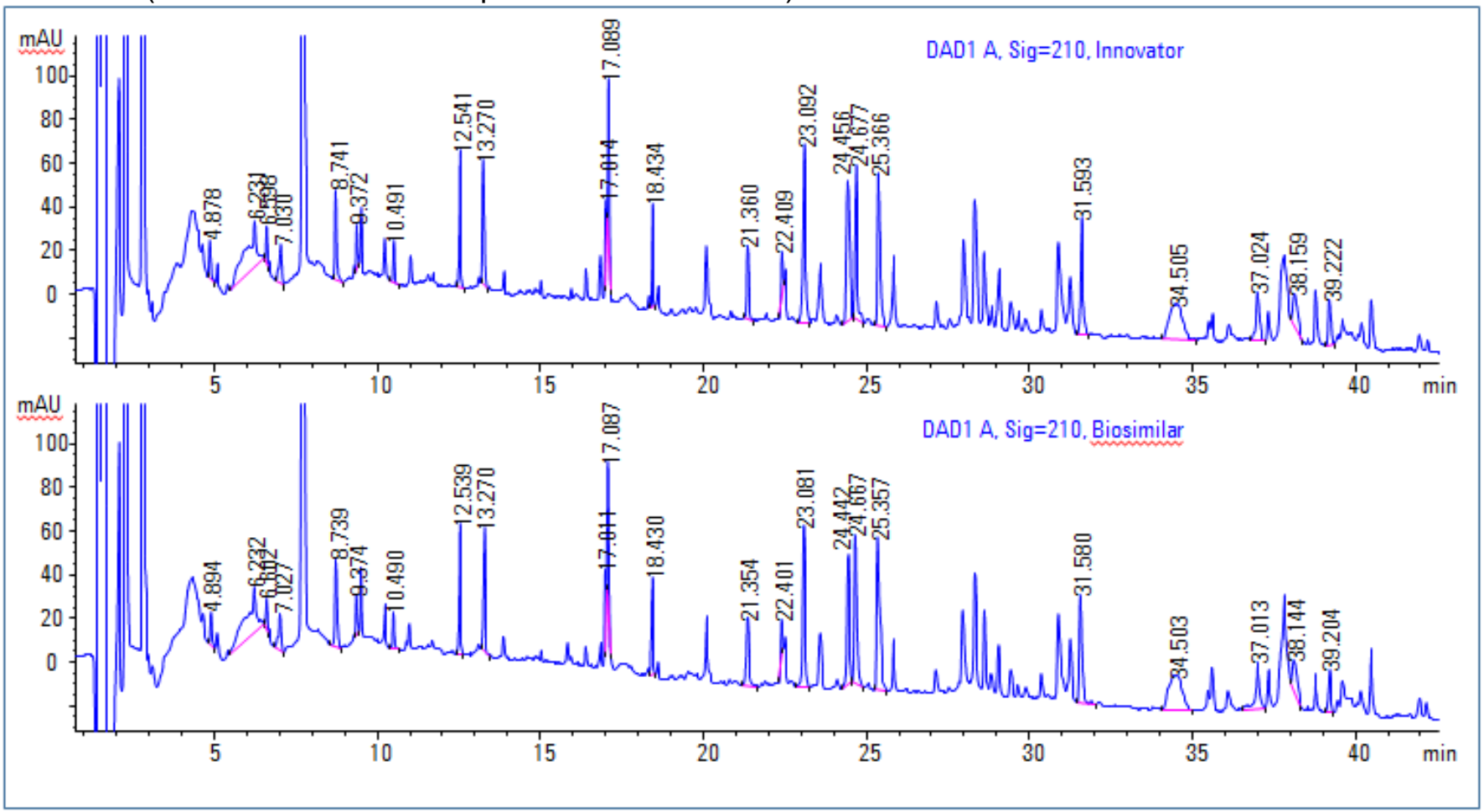


Zoom in of four representative peaks across the chromatogram to show separation reproducibility



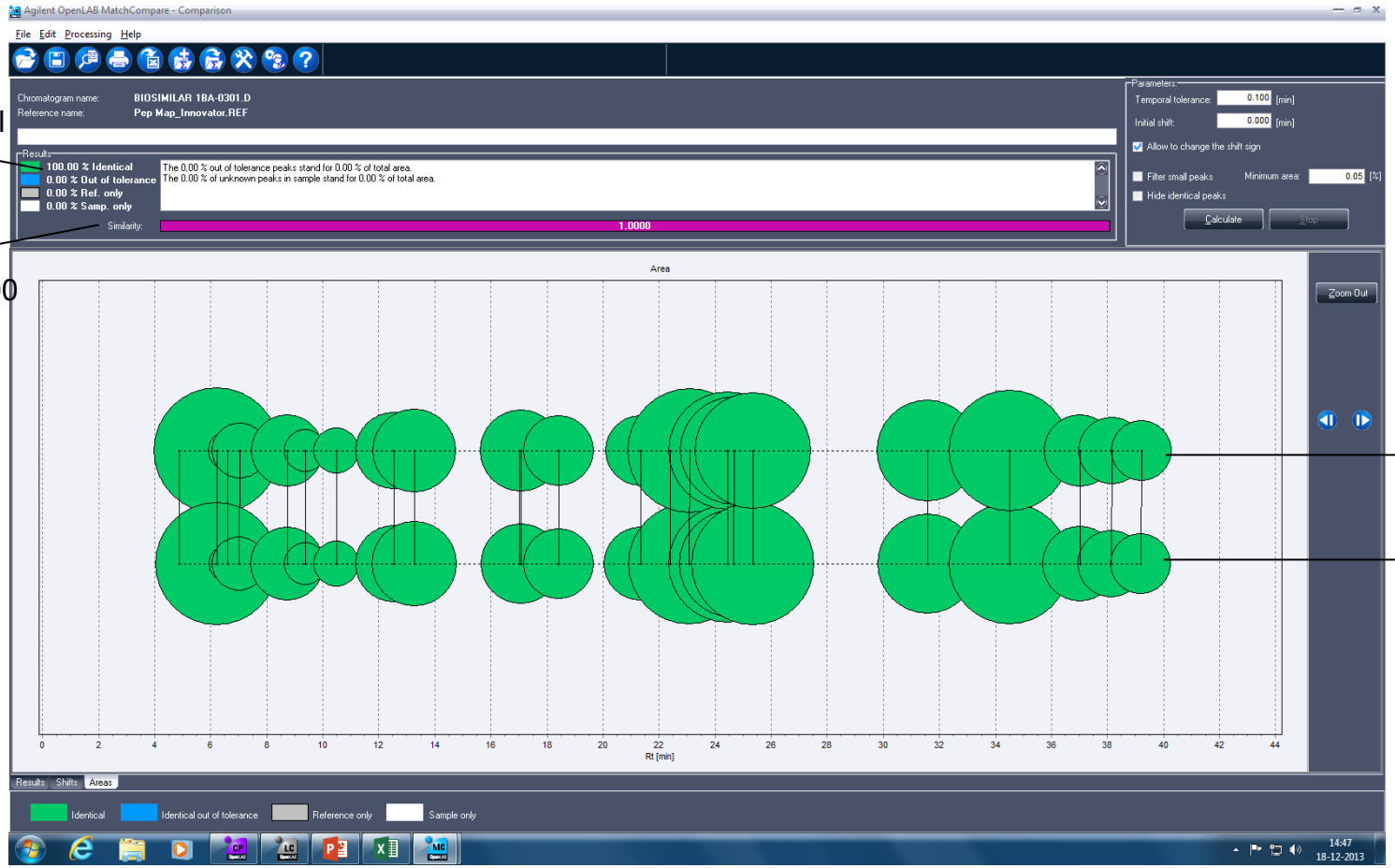
Peptide mapping of Biosimilar and innovator mAb

Comparison of peptide maps of innovator and biosimilar mAb using Agilent OpenLab Match Compare Software (Peaks selected for comparison are annotated)



Peptide mapping of Biosimilar and innovator mAb

Match Compare result



100% Identical

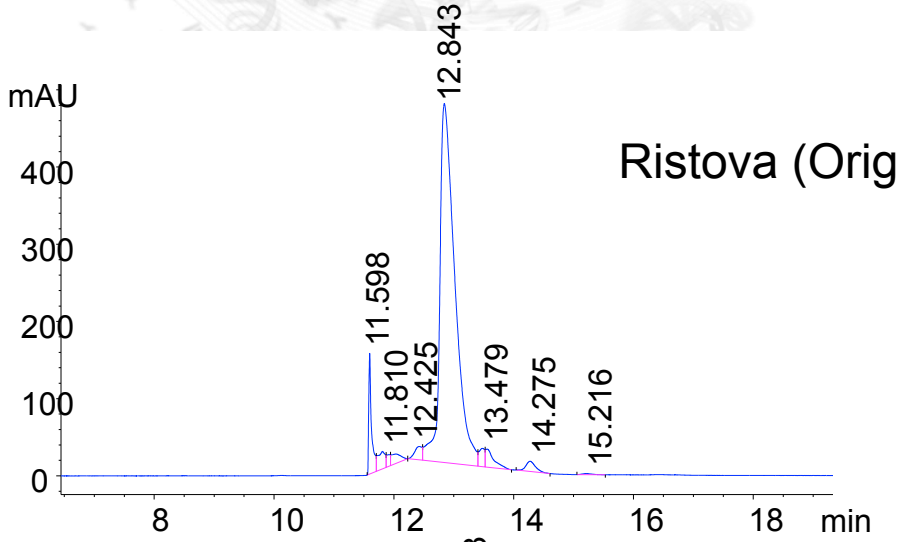
Similarity 1.000

Innovator

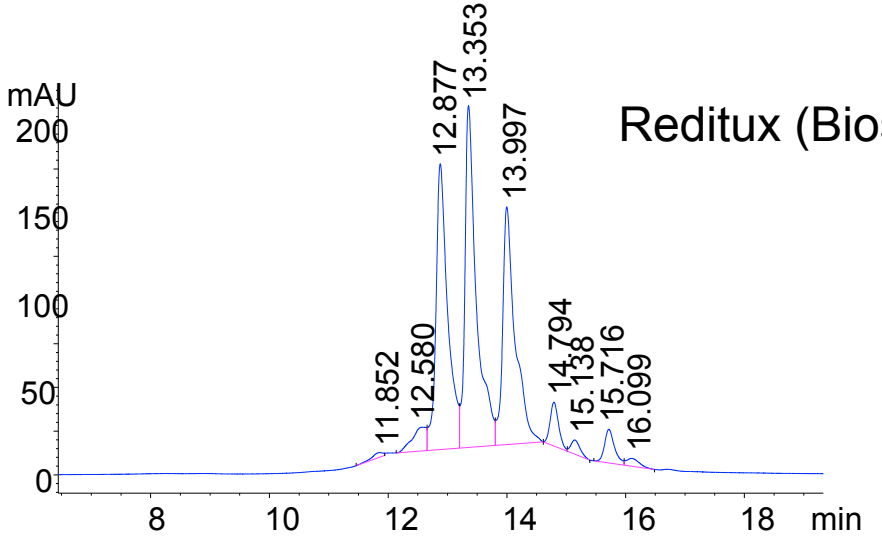
Biosimilar

Charge Variant Analysis of Biosimilar & Innovator

Agilent BioMAb WCX Column



Ristova (Originator)



Reditux (Biosimilar)

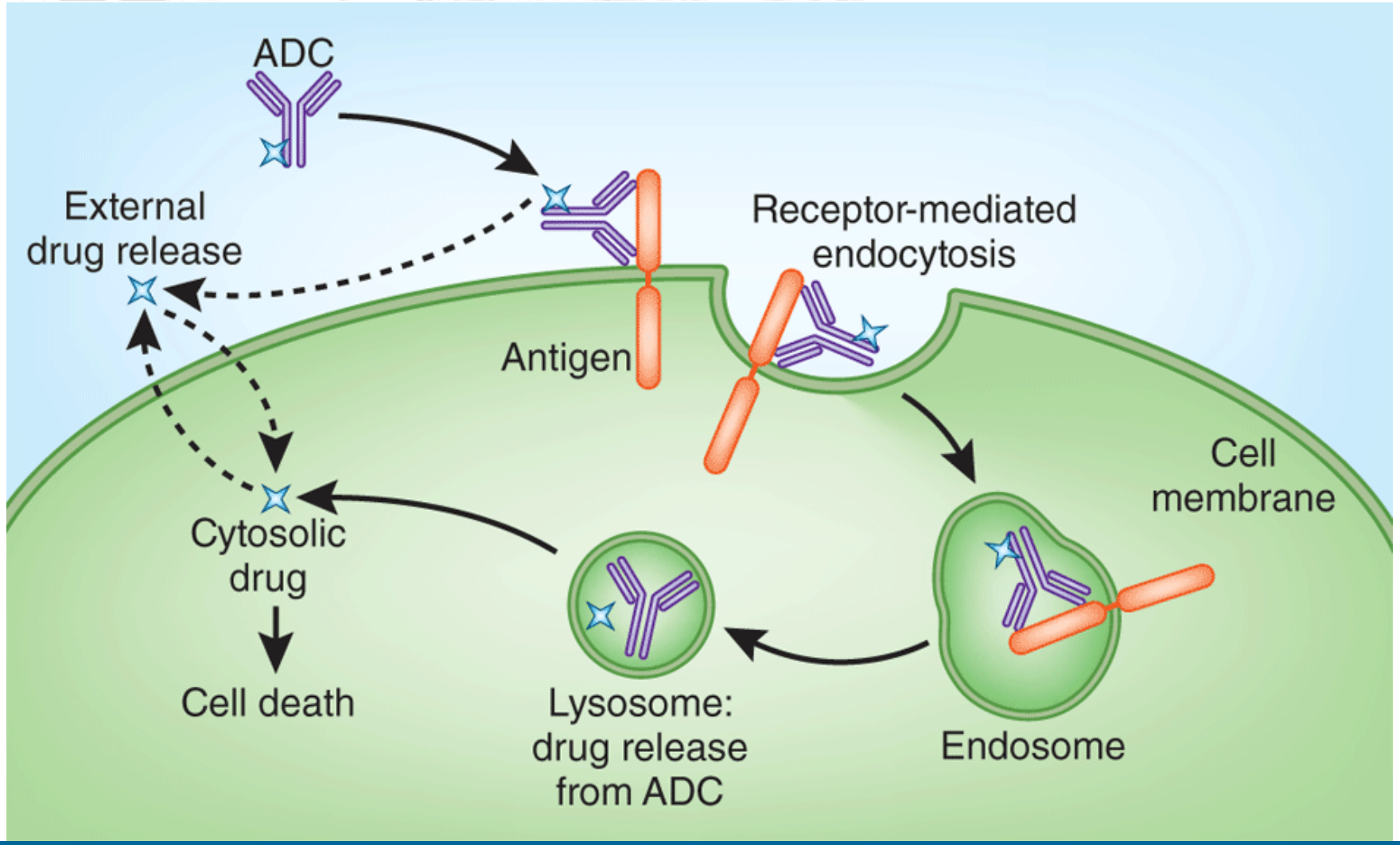
ANTIBODY DRUG CONJUGATE (ADC)

Rationale for Antibody Drug Conjugates (ADCs)

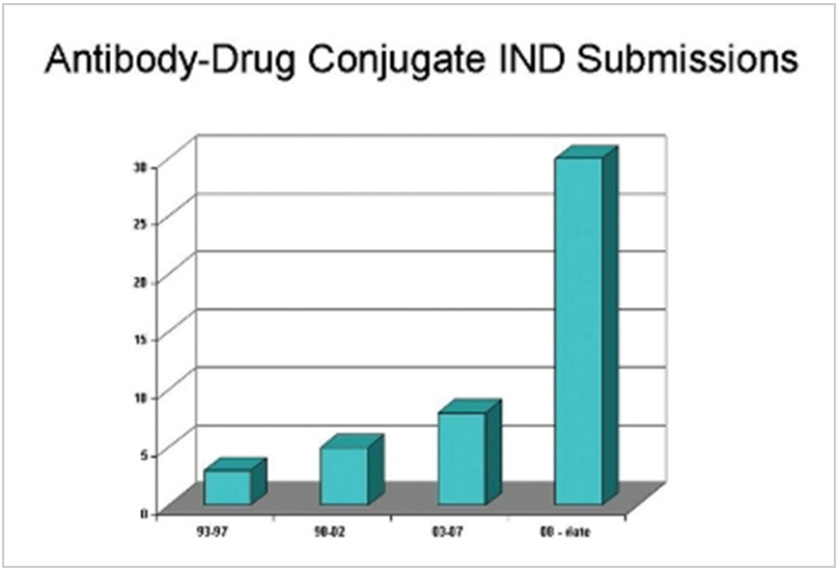
- Some small molecule drugs have high systemic toxicity, e.g. chemotherapy drugs used for cancer treatment
- Antibodies can target particular cells (e.g. antigen positive tumor cells) quite selectively
- Covalently linking antibodies to small molecule drugs can target the drug and reduce systemic toxicity
- With antibodies that have biological activity, conjugation may increase their effectiveness

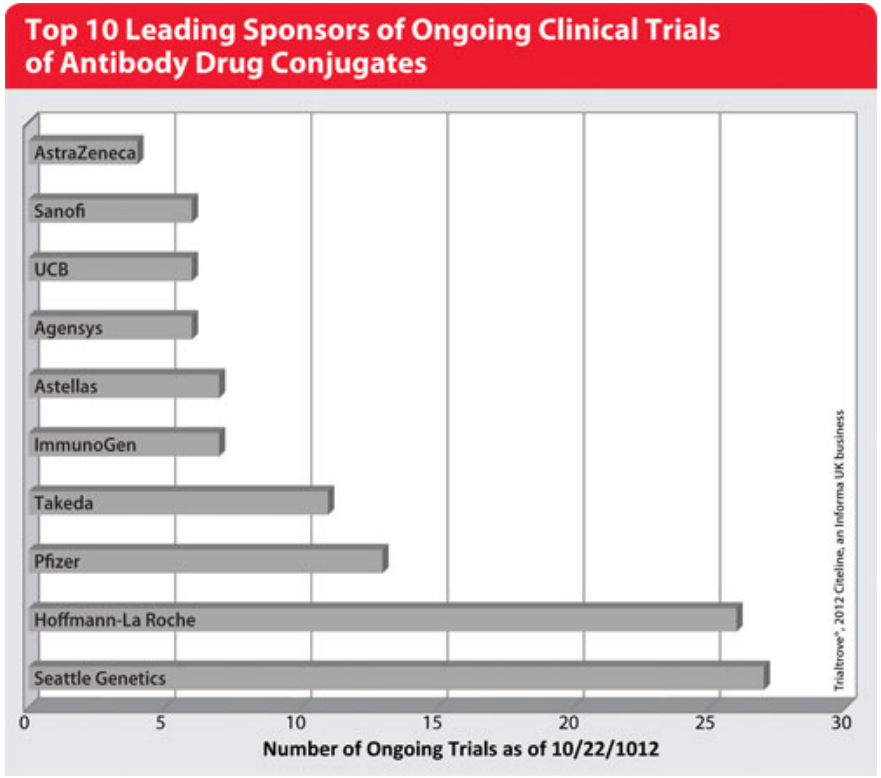
Antibody drug conjugates (ADCs)

Targeted cancer therapy



ADC
Surge in INDs





Antibody drug conjugate currently in clinical trials

Table 1. Antibody Drug Conjugates Currently in Clinical Trials

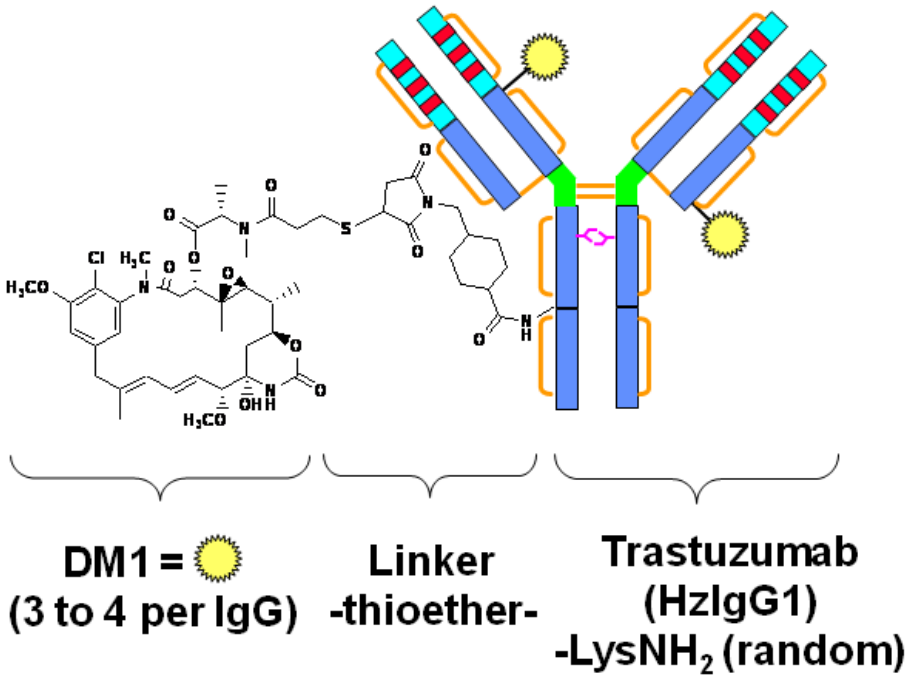
INN (Isotype)	Drug	Linker	Target	Indication	Sponsor	Clinical Stage (Phase)
Gemtuzumab ozogamicin (IgG4)	Calicheamycin	Hydrazone	CD33	AML	Pfizer (Wyeth)	MA2000 (with-drawn 2010)
Inotuzumab ozogamicin (IgG4)	Calicheamycin	Hydrazone	CD22	NHL	Pfizer (Wyeth)	III
Trastuzumab emtansine (IgG1)	DM1	Thioether	HER2	Breast ca	Genentech	III
Lorvotuzumab mertansine (IgG1)	DM1	Thioether	CD56	Myeloma	ImmunoGen	II
IMGN-388	DM4	Thioether	αV integrin	Solid tumors	Centocor	I
SAR3419	DM4	Disulfide	CD19	NHL	Sanofi-Aventis	I
BIIB015 (IgG1)	DM4	NA	Cripto	Breast cancer	Biogen Idec	I
BT-062 (IgG4)	DM4	Disulfide	CD138	Myeloma	Biotest	I
Brentuximab vedotin (IgG1)	vcMMAE	Valine-Citruline	CD30	HL	Seattle Genetics	III
Glembatumumab vedotin (IgG2)	vcMMAE	Valine-Citruline	GPNMB	Breast cancer, melanoma	Celldex	II
SGN-75	mcMMAF	Maleimidocapr.	CD70	NHL, RCC	Seattle Genetics	I
PSMA ADC	vcMMAE	Valine-Citruline	PSMA	Prostate cancer	Progenics	I
MEDI-547 (IgG1)	mcMMAF	Maleimidocapr.	EphA2	Solid tumors	MedImmune	I
ASG-5ME	vcMMAE	Valine-Citruline	SLC44A4	Pancreatic cancer	Agensys	I
MN	Auristatin	NA	MN	Cancer	Bayer Schering	I
MDX-1203	Duocarmycin	Dipeptide	CD70	NHL, RCC	BMS (Medarex)	I

← approved

← on market now

Antibody drug conjugates (ADCs)
Design

T-DM1 (Genentech)

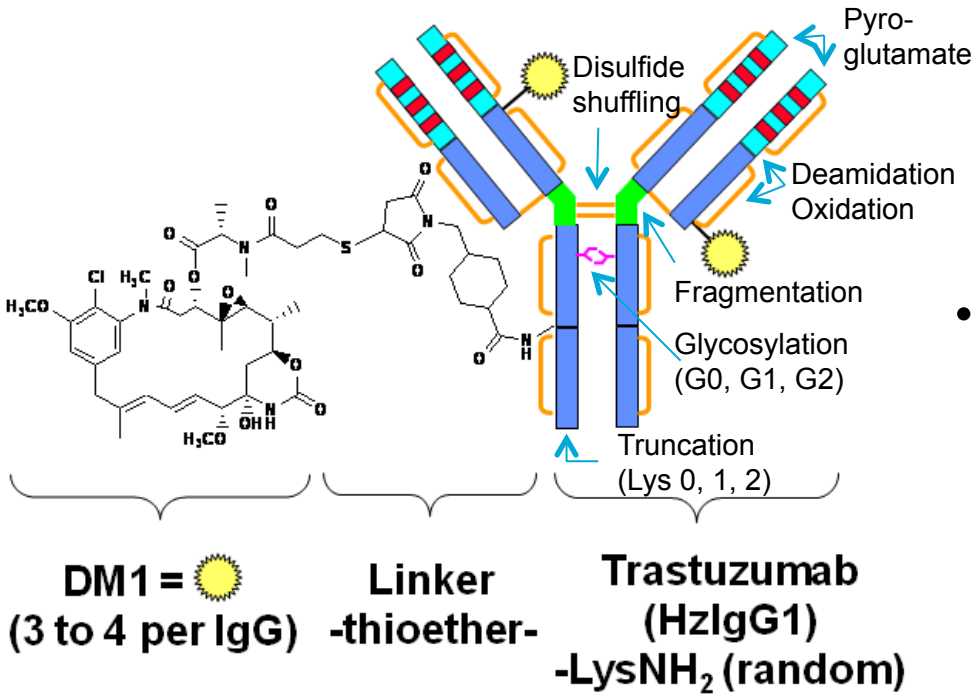


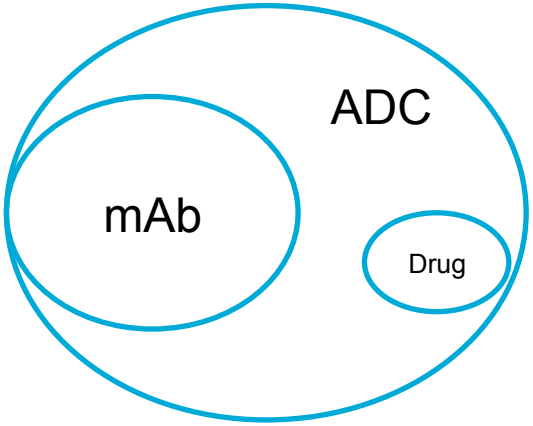
Components:

- Antibody
  - Targeted recognition
  - Abundant target expression & internalization
- Drug
  - Highly potent
  - Validated mechanism of action (microtubule inhibition, DNA damage
- Linker
  - Stable in plasma
  - Labile upon internalization to release drug

Heterogeneity in ADCs
Complexity

T-DM1 (Genentech)

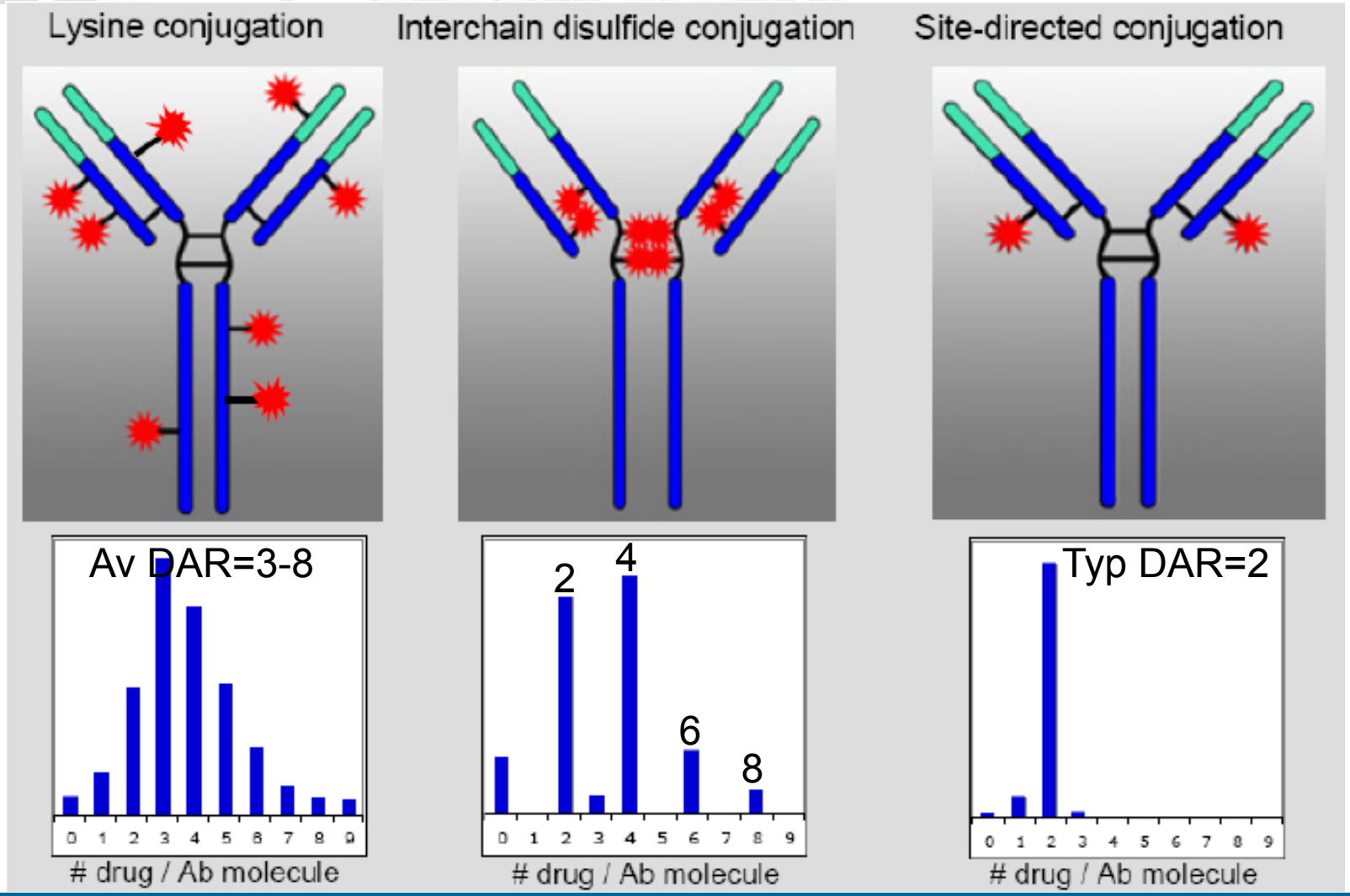




- Heterogeneity in ADC
  - More complex than mAbs alone
  - Dependent on linker & payload stability (hydrolysis, degradation, etc)
  - Dependent on the conjugation chemistry

Heterogeneities in ADCs from conjugation

Dependent on conjugation chemistry



Analytical complexity and heterogeneity

- Conjugation introduces heterogeneity on top of that already present in the antibody

- Additional assays required beyond those for an antibody due to presence of cytotoxic agent, e.g.
  - Drug to antibody ratio (DAR)
  - Amount of free and bound cytotoxic agent

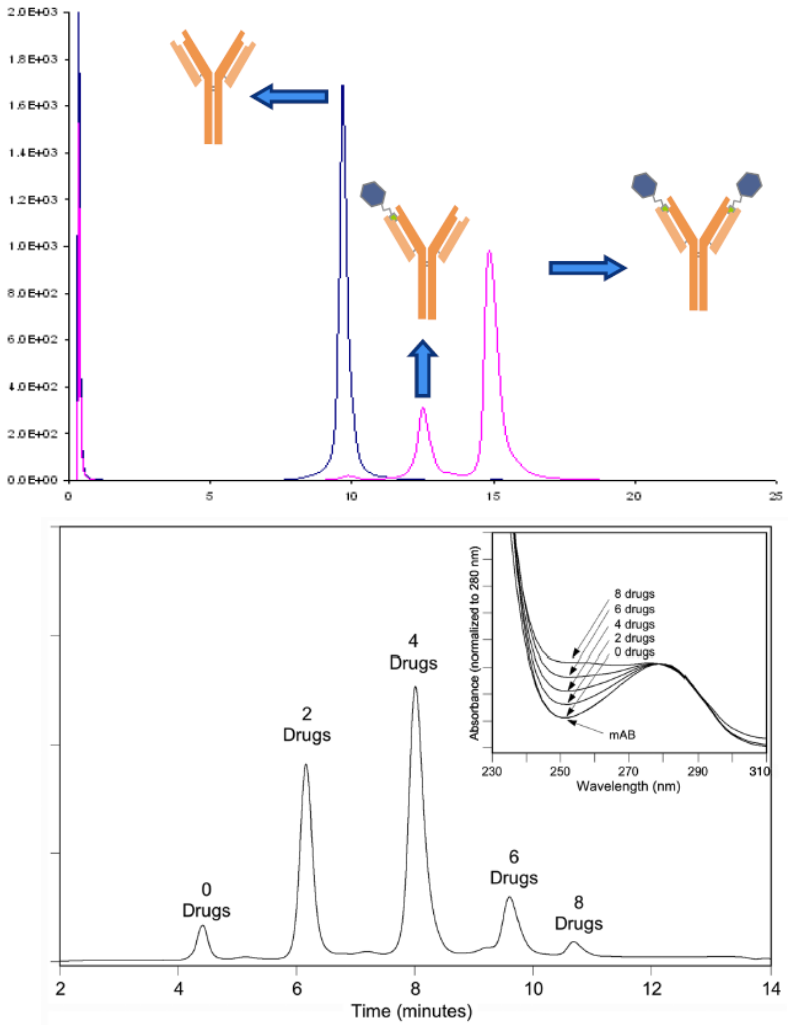
Regulatory considerations in developing antibody drug conjugates

- Regulatory Jurisdiction
  - A conjugate is composed of small molecule components (linker and cytotoxic agent) and an antibody
  - What are the roles of divisions with expertise in the components (e.g. ONDQA and DMA)?
  - With divided responsibility, how is review and interaction with the sponsor coordinated?
- Classification of components (Antibody, Linker, Cytotoxic Agent) used to manufacture conjugates
  - Is existing terminology appropriate (e.g. starting material, intermediate, API)?
  - What are the consequences for testing, validation, process changes?

Key quality attributes & methods for ADC

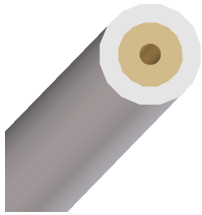
Quality attributes	Assays	
Identity	Intact Mass, Peptide Mapping, Sequence	mAb + ADC
Size heterogeneity (aggregates)	SDS-PAGE, SEC, MALS, MS	mAb + ADC
Charge heterogeneity	IEF, CEX	mAb + ADC
PTMs	LC/MS	mAb + ADC
Drug load (DAR)	UV, HIC, HPLC	ADC
Drug load distribution	HIC, MS	ADC
Residual drug	ELISA, HPLC	ADC
Potency (for drug)	Cytotoxicity	ADC
Potency (for mAb)	Antigen binding ELISA	mAb + ADC

Drug to antibody ratio (DAR) by HIC



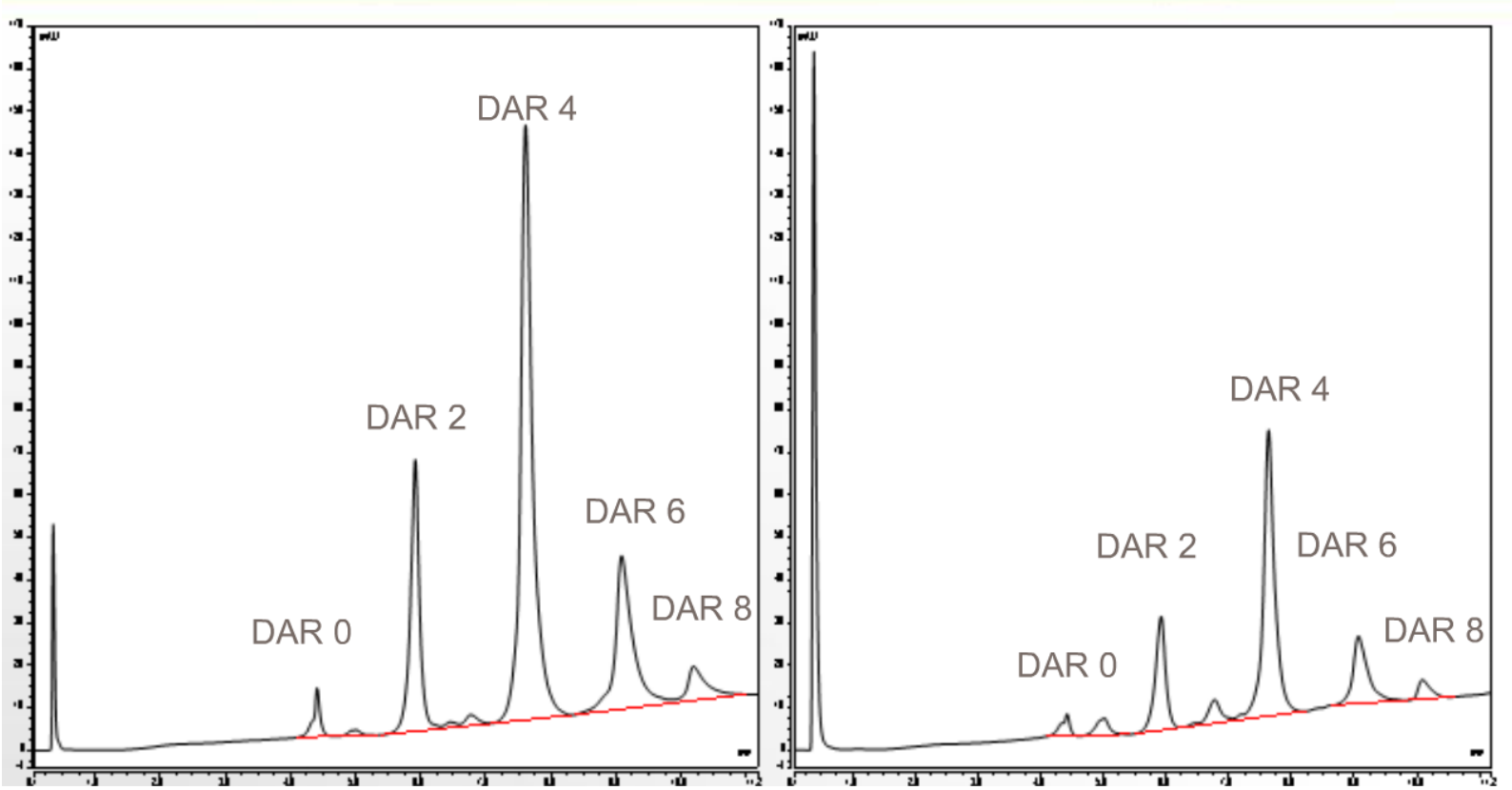
- HIC is gentle and does not disrupt non-covalent interactions. This retains MAb structures lacking normal disulfide bonds as found in some conjugates
- Typical HIC eluents
  - A: 2.0 M $(NH_4)_2 SO_4$ in 0.1M NaH_2PO_4, pH 7.0; B: 0.1 M NaH_2PO_4, pH 7.0

BIO inert



- Metal cladded PEEK design
- 600 Bar

DAR-HIC stability studies

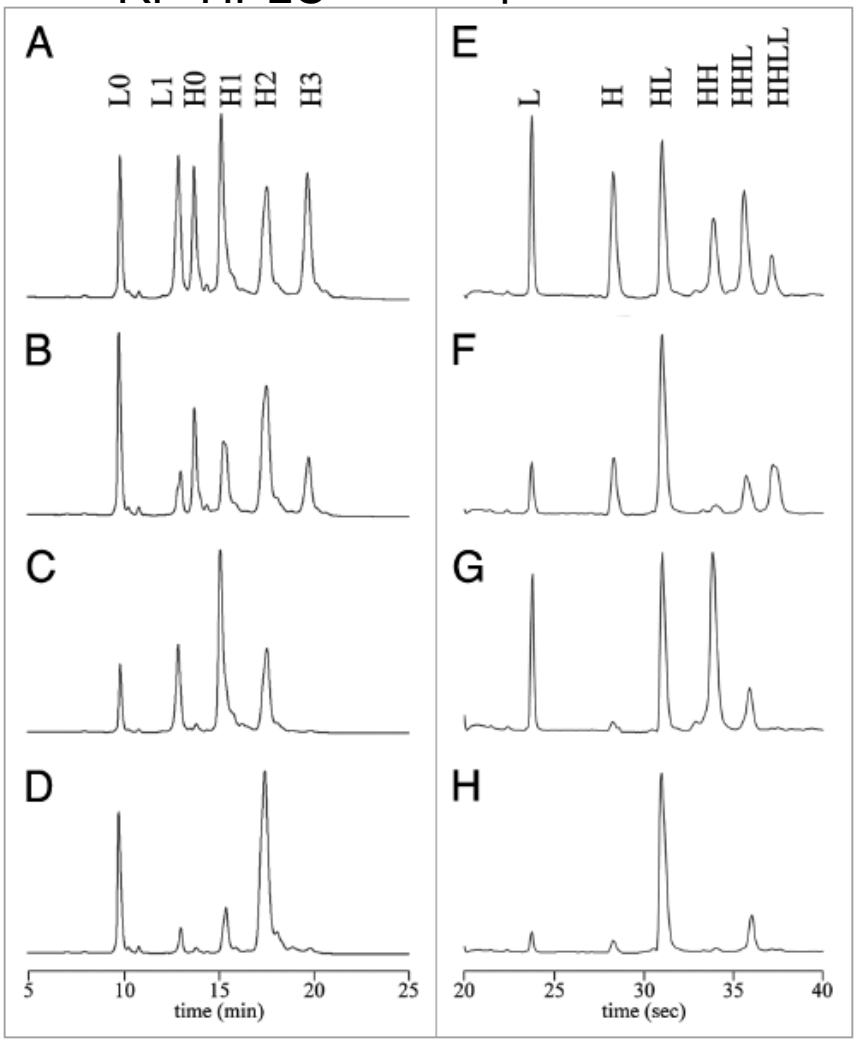


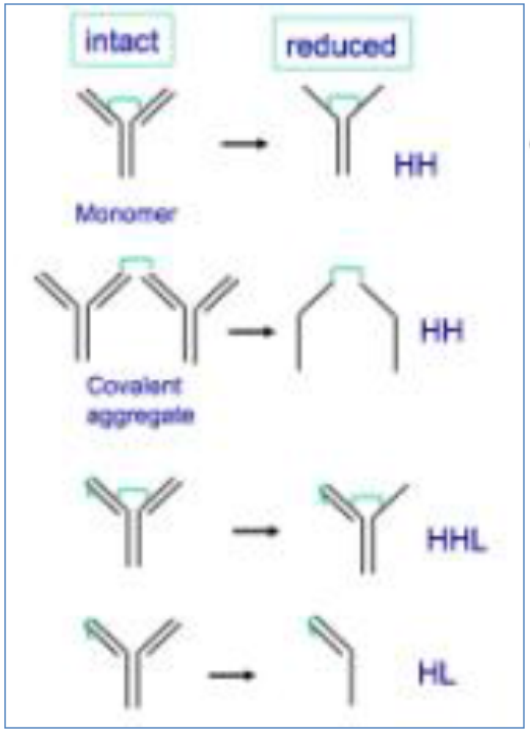
- Lyophilized sample - T0
- Solution sample – 2 weeks @ 40C

Analysis of reduced ADC fragments

RP-HPLC

Bioanalyzer (SDS)
TapeStation





Bioanalyzer (SDS)

TapeStation

Figure 4. (A–D) Reversed-phase HPLC analysis of DTT-reduced conjugates produced using different reduction/reoxidation protocols. (E–H) Analysis of the same conjugate samples in (A–D), under non-reducing conditions, using the Agilent Bioanalyzer™, a silicon chip based system for capillary electrophoresis in the presence of SDS (CE-SDS).[12] Adapted with permission from Sun MM, Beam KS, Cerveny CG, Hamblett KJ, Blackmore RS, Torgov MY, et al. Reduction-alkylation strategies for the modification of specific monoclonal antibody disulfides.[12]

A Wakankar, Y Chen, Y Gokarn & F Jacobson (Genentech)

Size variant analysis of conjugates

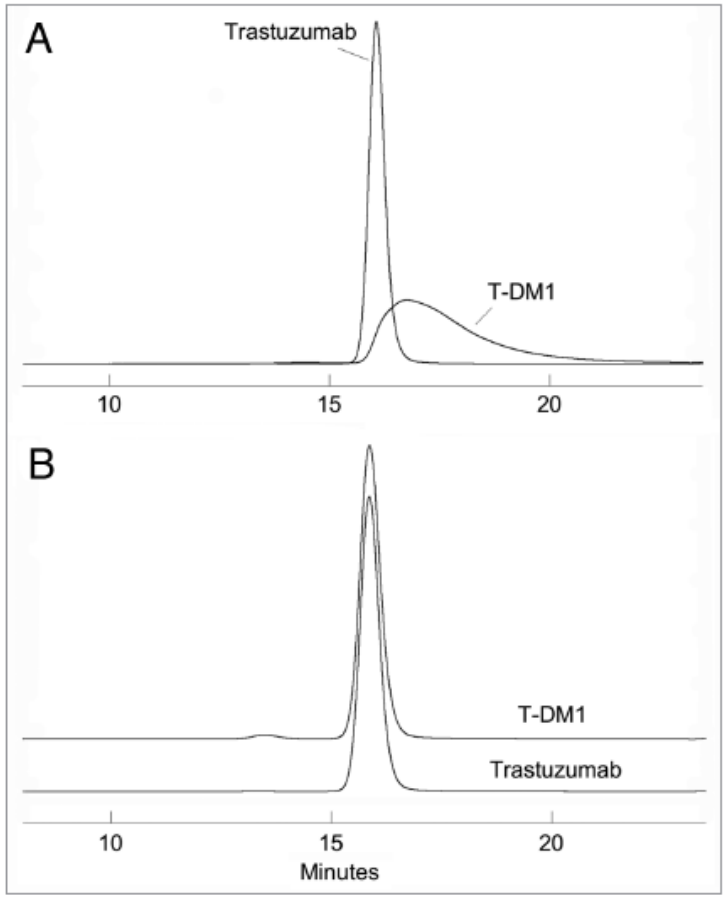


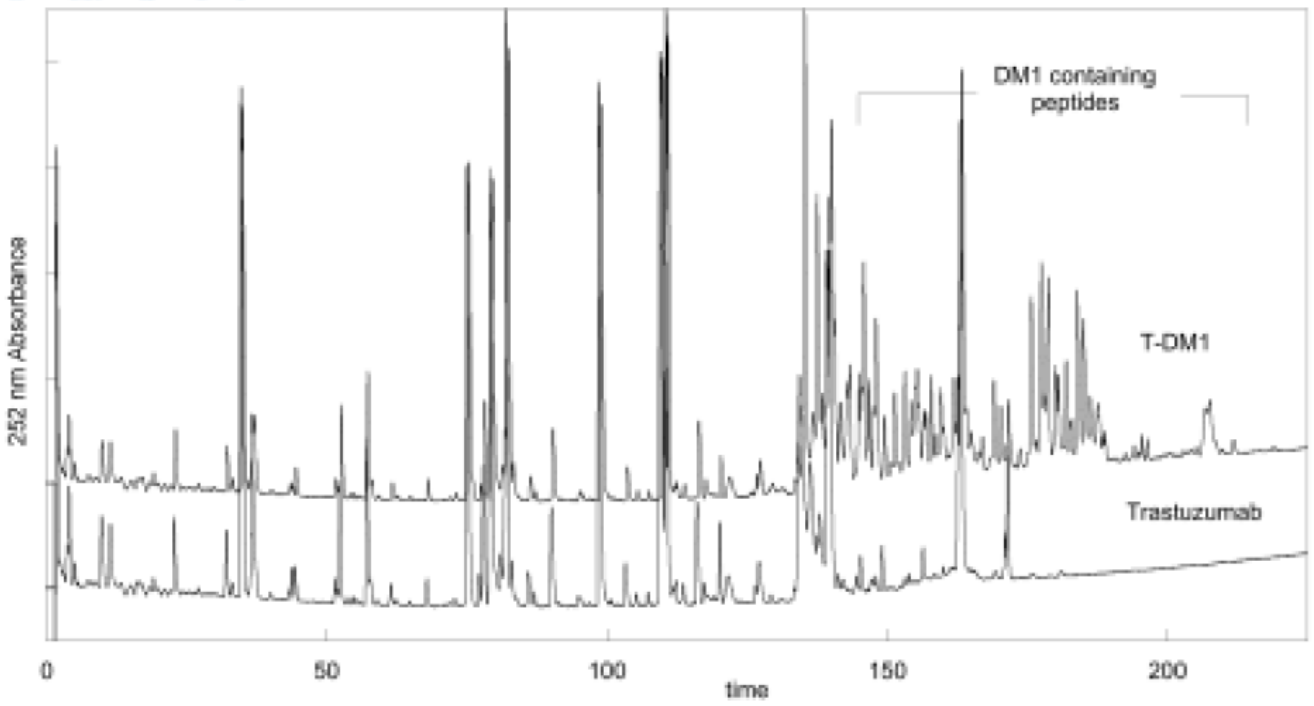
Figure 5. SEC analysis on a TSK 3000SW$_{XL}$ column run at 0.5 mL/min and monitored by 280 nm absorbance. (A) Mobile phase is 0.2 M KPi and 0.25 M KCl, pH 6.95. (B) 85% KPi/KCl mobile phase; 15% 2-propanol.

BIO inert

+ Bio MAb SEC-3/5

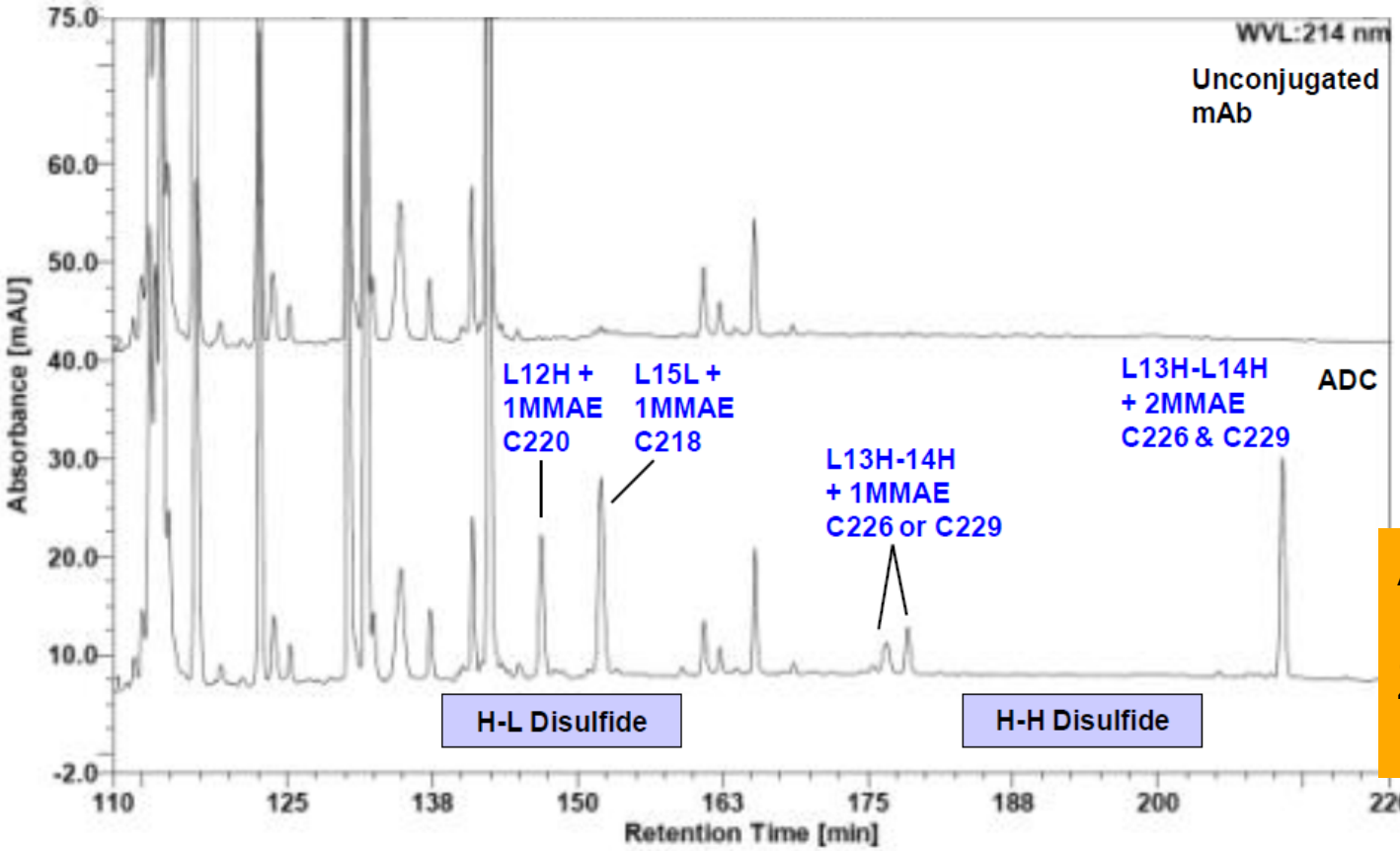
Peptide mapping of ADC

- Enzymatic cleavage of ADCs can be used to identify drug-containing peptides
  - Peptides labeled with a hydrophobic drug would be expected to elute later than their unmodified forms in the RP-HPLC chromatogram due to increased retention by the column
  - Trastuzumab has 88 lysines for possible linkage.DM1 chromophore (λmax =252nm) can be used to identify peptides containing bound drug. Peptides (~60 new peaks) with drug elute at end of gradient



Agilent Solutions:
1260 Bio or 1290
Zorbax & AdvanceBio
Peptide Mapping

Simple peptide map; Cys-linked ADC

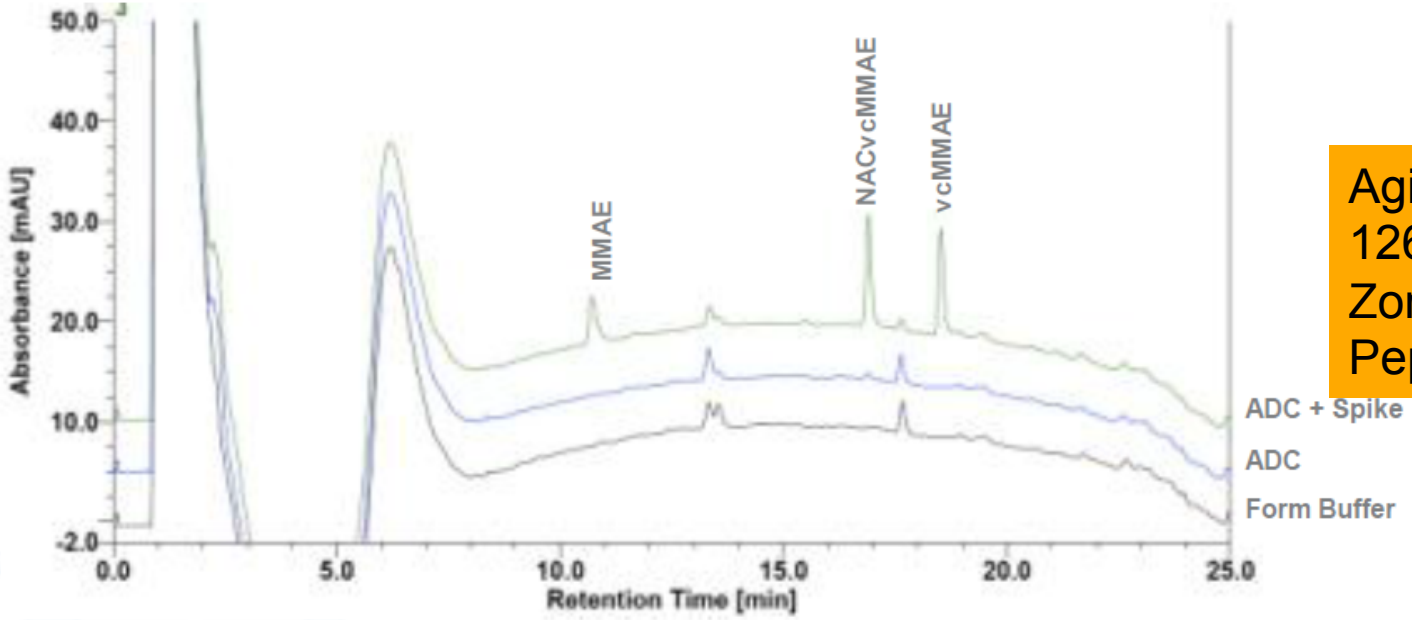


Agilent Solutions:
1260 Bio or 1290
Zorbax & AdvanceBio
Peptide maping

- 4 major sites of vcMMAE conjugation at interchain disulfide bonds:
  - Cys 220 and Cys 218 (H-L)
  - Cys 226 and Cys 229 (H-H)

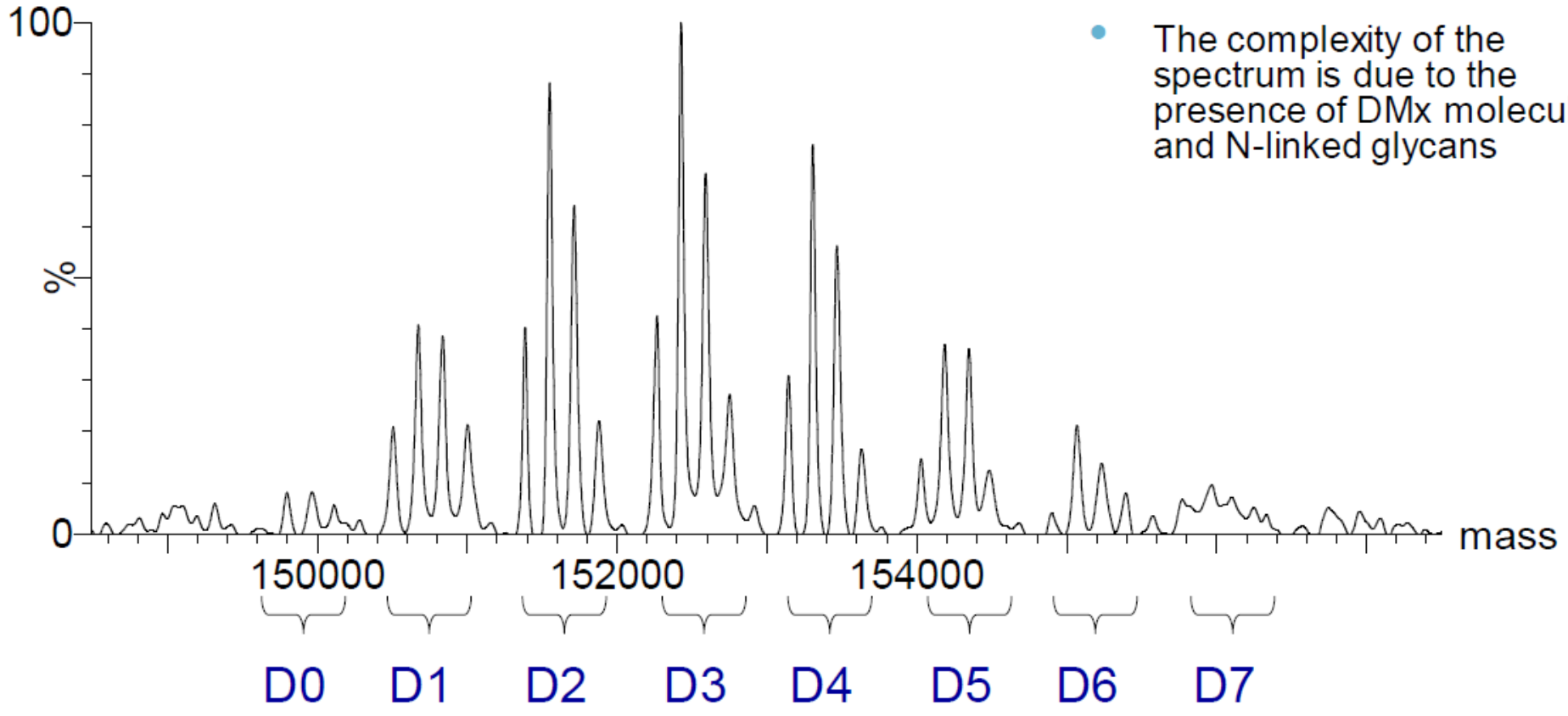
Free drug analysis by RP-HPLC

- Free drug species are very low in cysteine-linked ADCs
- Barely detectable increases in free drug during storage, even at elevated temperatures
- Lysine linked conjugates (e.g. T-DM1) show some time dependent release of drug-linker from side-reactions w/other amino acids



Agilent Solutions:
1260 Bio or 1290
Zorbax & AdvanceBio
Peptide maping

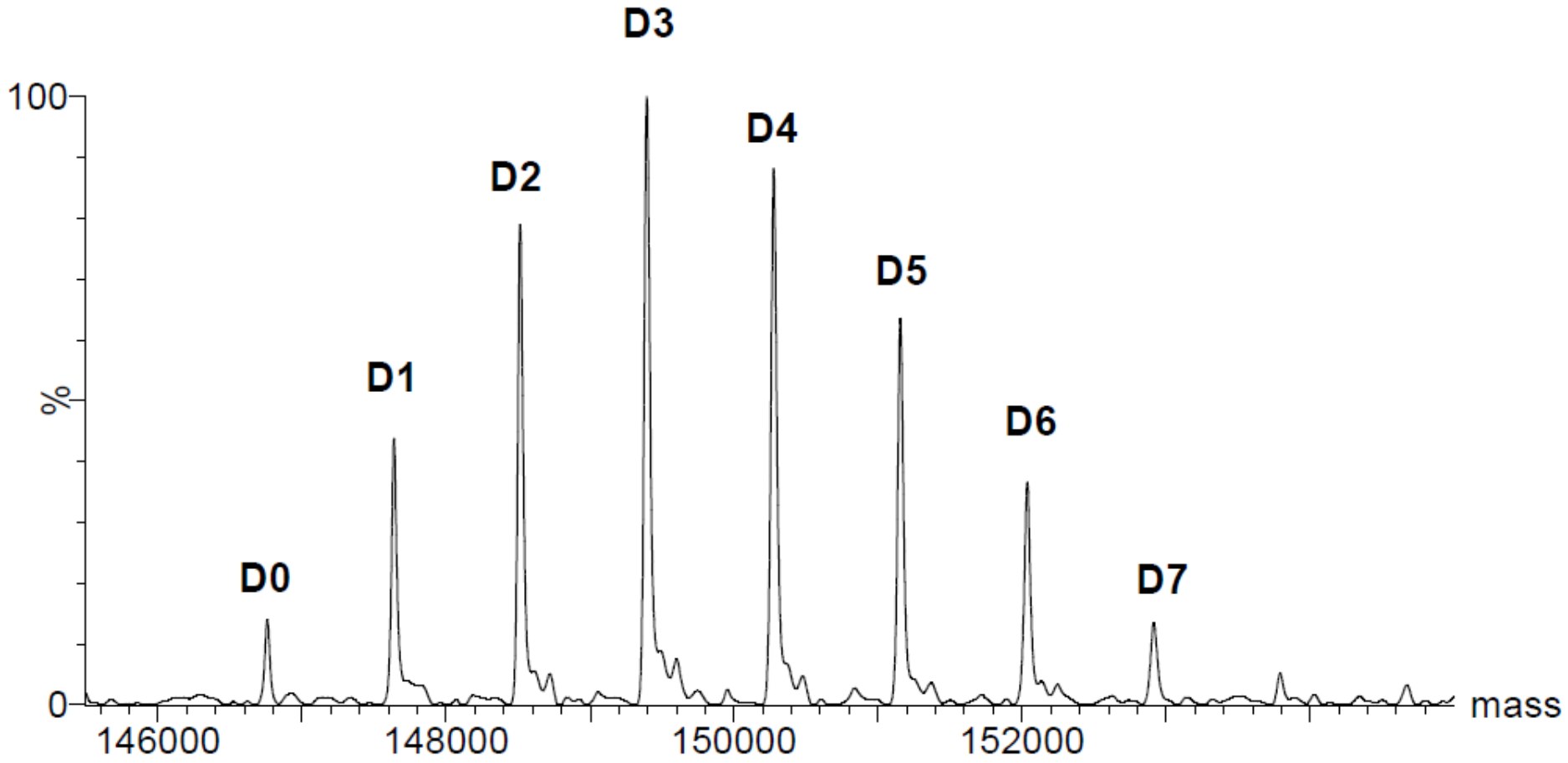
Drug distribution & location by LC/MS



- The complexity of the spectrum is due to the presence of DMx molecules and N-linked glycans

Dn represents the number of DMx molecules per MAb

Deglycosylating the conjugate reveals heterogeneity due to conjugation



Dn represents the number of DMx molecules per MAb

ADC publication using Agilent QTOF

Seattle Genetics

analytical chemistry

Article

pubs.acs.org/ac

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Native Intact Mass Determination of Antibodies Conjugated with Monomethyl Auristatin E and F at Interchain Cysteine Residues

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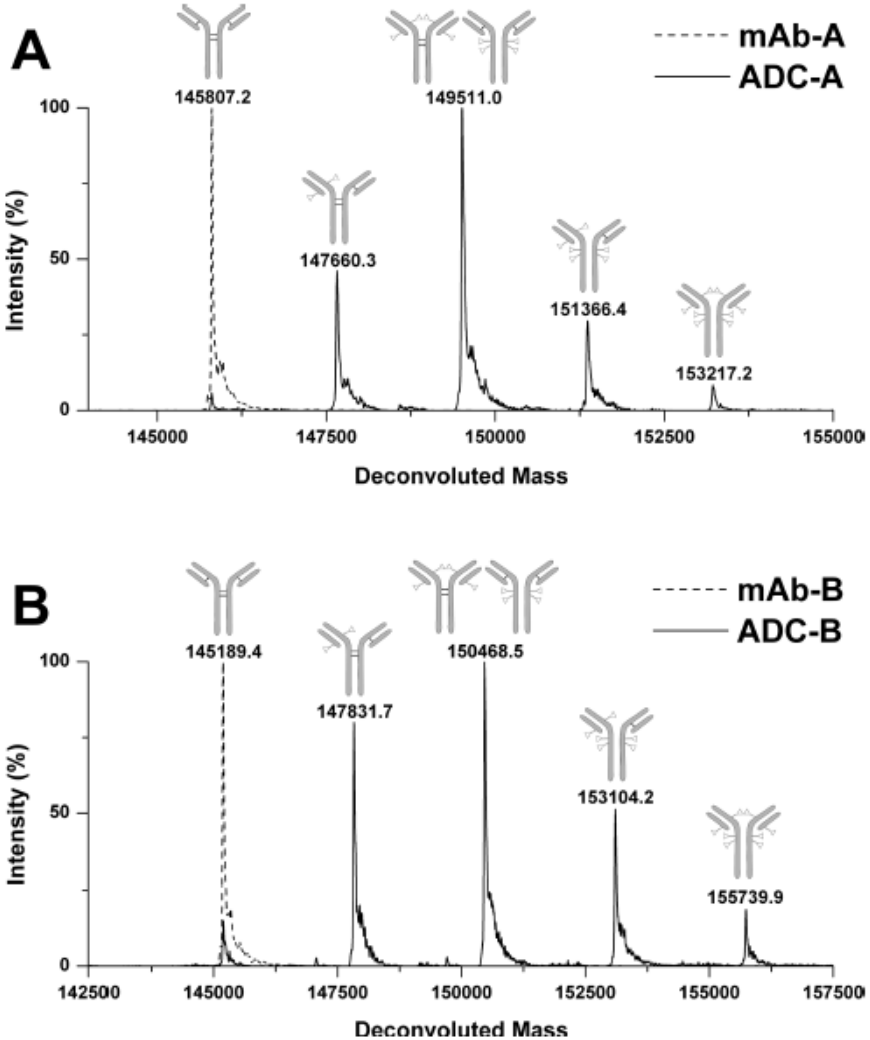
Mass Spectrometry. Mass spectral data for mAbs and ADCs was acquired on an Agilent 6510 QTOF (Agilent, Santa Clara, CA) in positive electrospray ionization (ESI) mode in the range 1000–8000 *m/z*. The drying gas temperature was 350 °C, and flow rates for the drying gas and the nebulizer gas pressure were 12 L/h and 35 psi, respectively. The capillary, fragmentor, and octupole rf voltages were set at 5000, 450, and 750, respectively. The raw data was converted to zero charge mass spectra with a maximum entropy deconvolution algorithm within the MassHunter workstation software version B.03.01.

Native MS Conditions

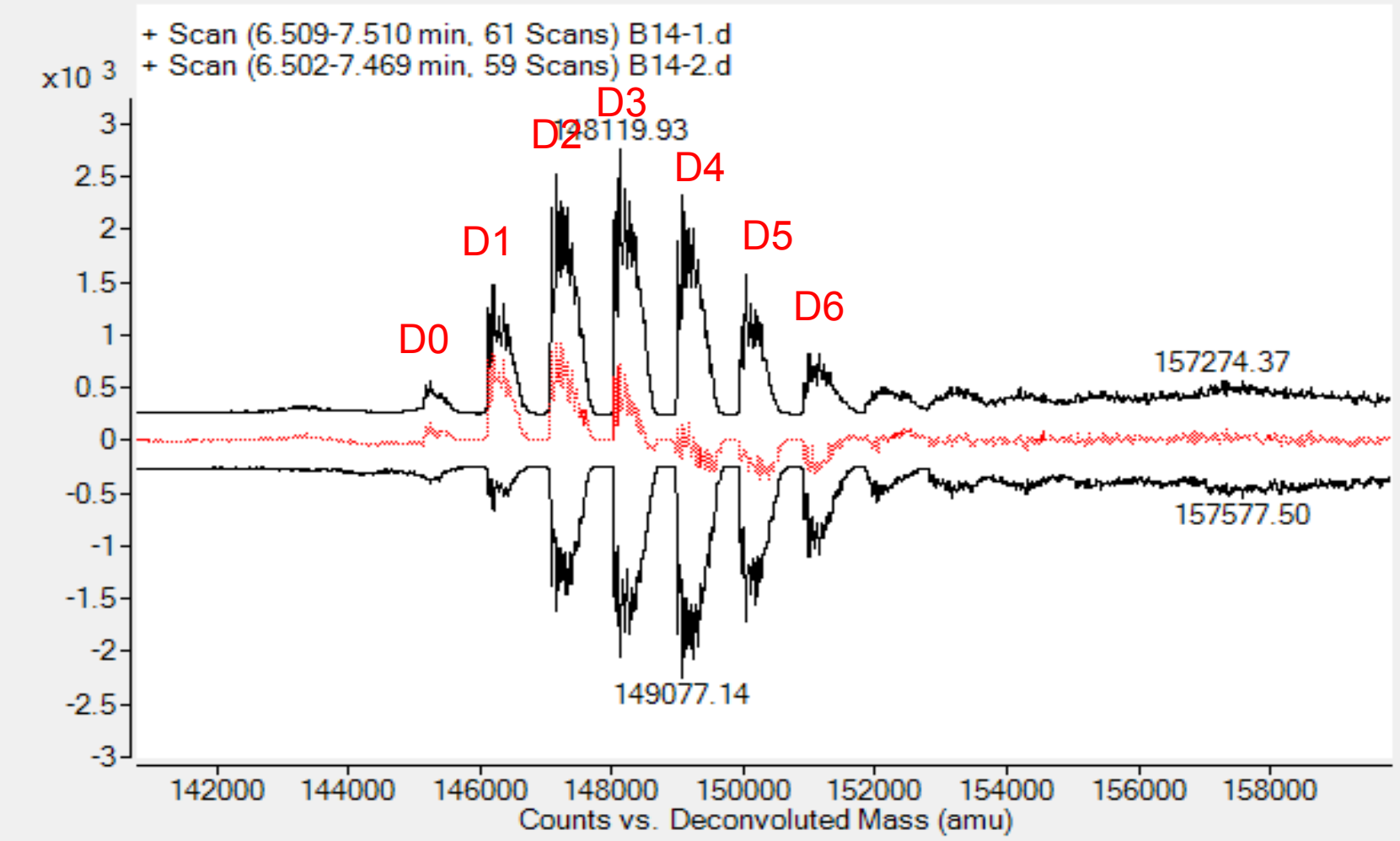
UHPLC: Agilent 1290 Infinity Binary pump, well plate autosampler, thermostatted column compartment, 1200 binary pump

Column:	PolyLC PolyHYDROXYETHYL A, 1 x 50 mm, 5 µm, 300 Å
Column temperature:	23 ºC
Injection volume:	1-5 µL
Autosampler temp:	4 ºC
Needle wash:	flushport (50% MeOH in H2O), 10 seconds
Mobile phase:	**A = 200 mM Ammonium Acetate (Native)**
	B = 0.2% Formic Acid, 30% Acetonitrile in Water (Denaturing)
Flow rate:	**0.050 mL/min**
Gradient:	**ISOCRATIC**
Stop time:	**5.00 min**

Mass measurement of a deglycosylated mcMMAF conjugate ADC



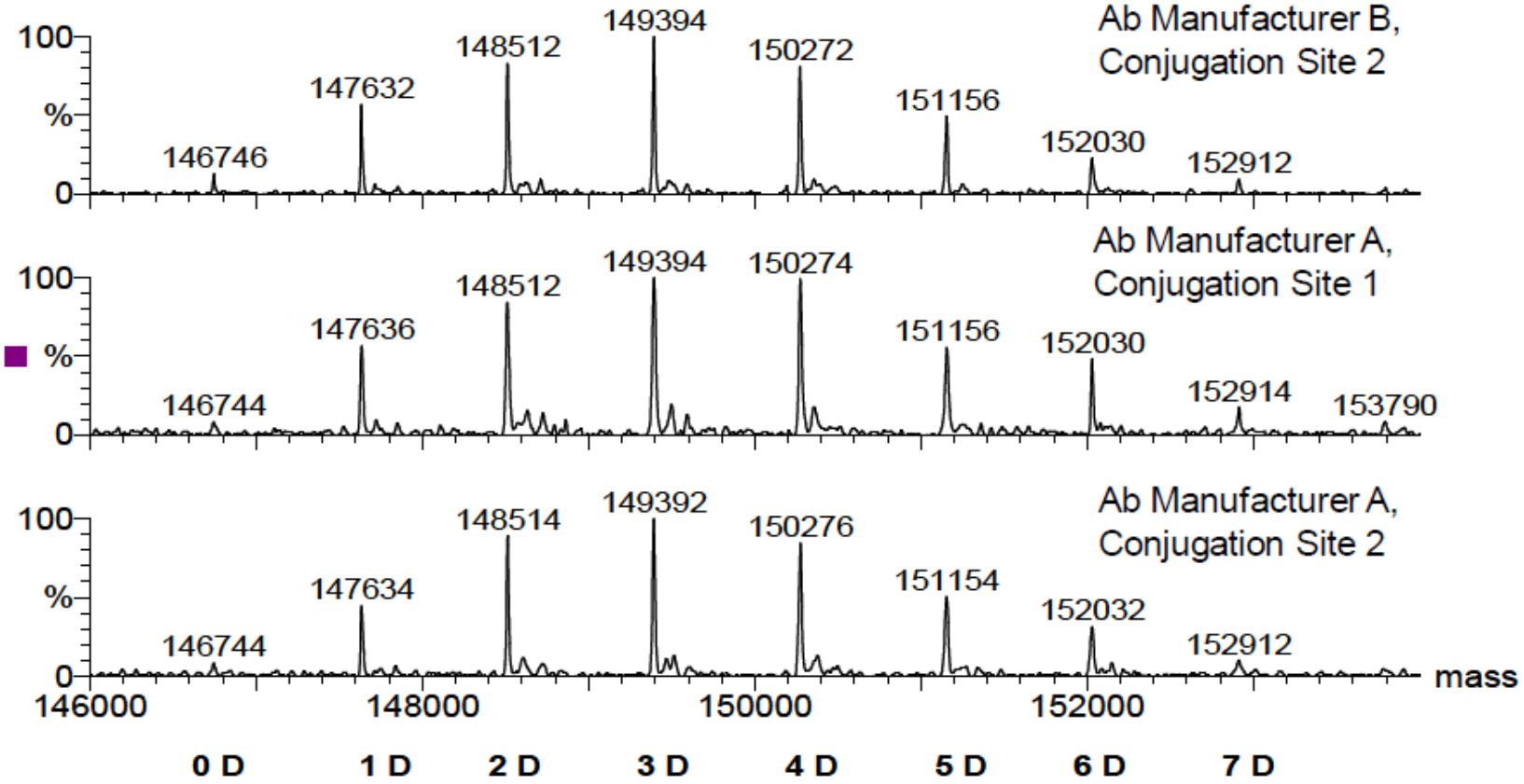
Comparing Two Batches of ADC



Mass distribution profile is a good test of process consistency

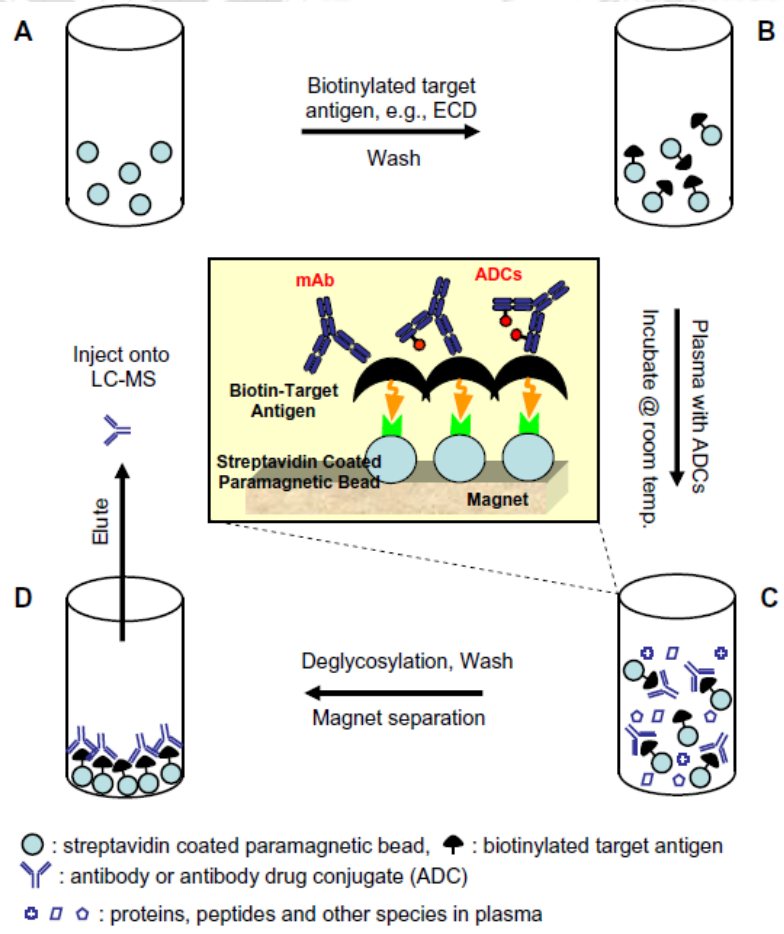
- UV based assay gives average drug loading
  - MS provides orthogonal verification
- MS also provides information on relative amounts of antibodies loaded with 1, 2, 3, 4 etc. drugs (without prior separation)
- MS also provides information on unconjugated antibody
- MS can detect other conjugation products, e.g.
  - Cross linked species
  - Species with linker attached but no drug

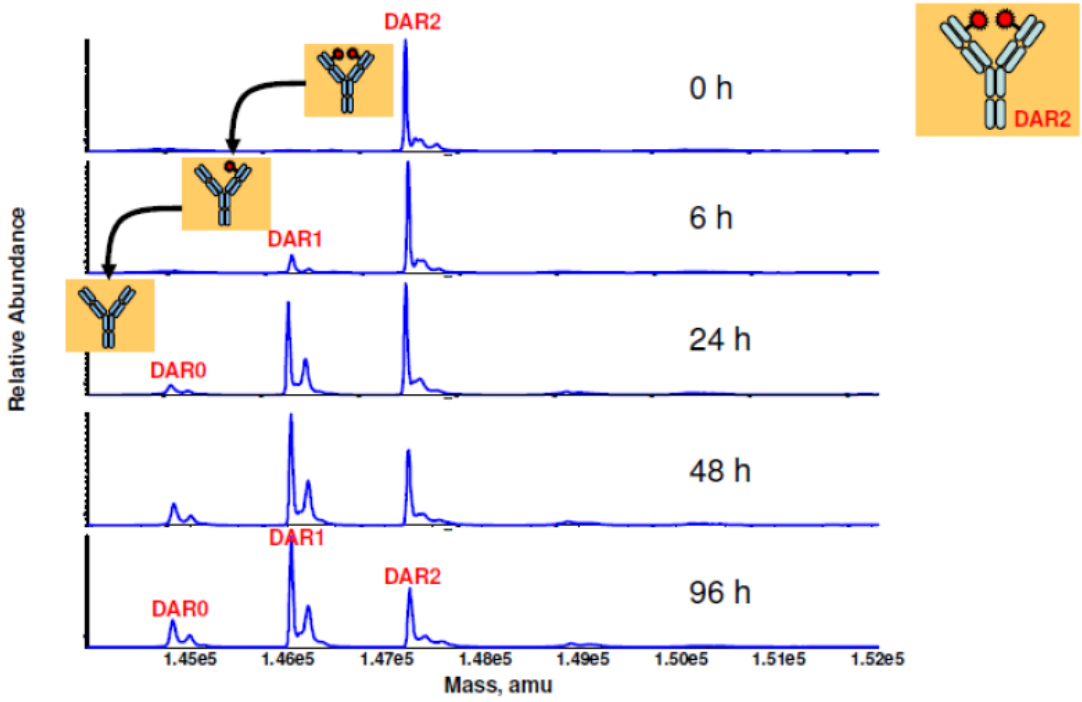
Mass distribution profile shows comparability after process changes



Other analytical methods also show these batches are comparable

in vivo Stability assay for ADCs & their metabolites in serum (PK) by affinity capture LC-MS





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AssayMAP Bravo Platform

Summary

- Interest in Antibody Drug Conjugates is high and growing
- Conjugates are analytically challenging due to their complexity and heterogeneity
- There is regulatory complexity with conjugates due to split review responsibilities within the FDA
- Agilent has solution to analyze ADCs
  - Bio-Inert 1260 for HIC application for DAR
  - 1290 with RP columns for peptide mapping to located conjugation sites
  - LC-QTOF/TOF can be used for mass distribution profile
    - Heterogeneity due to the conjugate can be distinguished from that of the Antibody
  - AssayMAP/Encore automation solution can be leverage to capture ADC for PK/PD and conjugation stability studies in plasma
  - Bioanalyzer and TapeStation can be used to monitor ADC fragments & oxidation-reduction products during conjugation process

Thank you for your attention!

Q&A