REGULATORY CONSIDERATIONS WHEN DEVELOPING RELEVANT AND SUSTAINABLE ANALYTICAL TECHNOLOGIES

PATRICK SWANN

March 9, 2018
AT Europe - Barcelona

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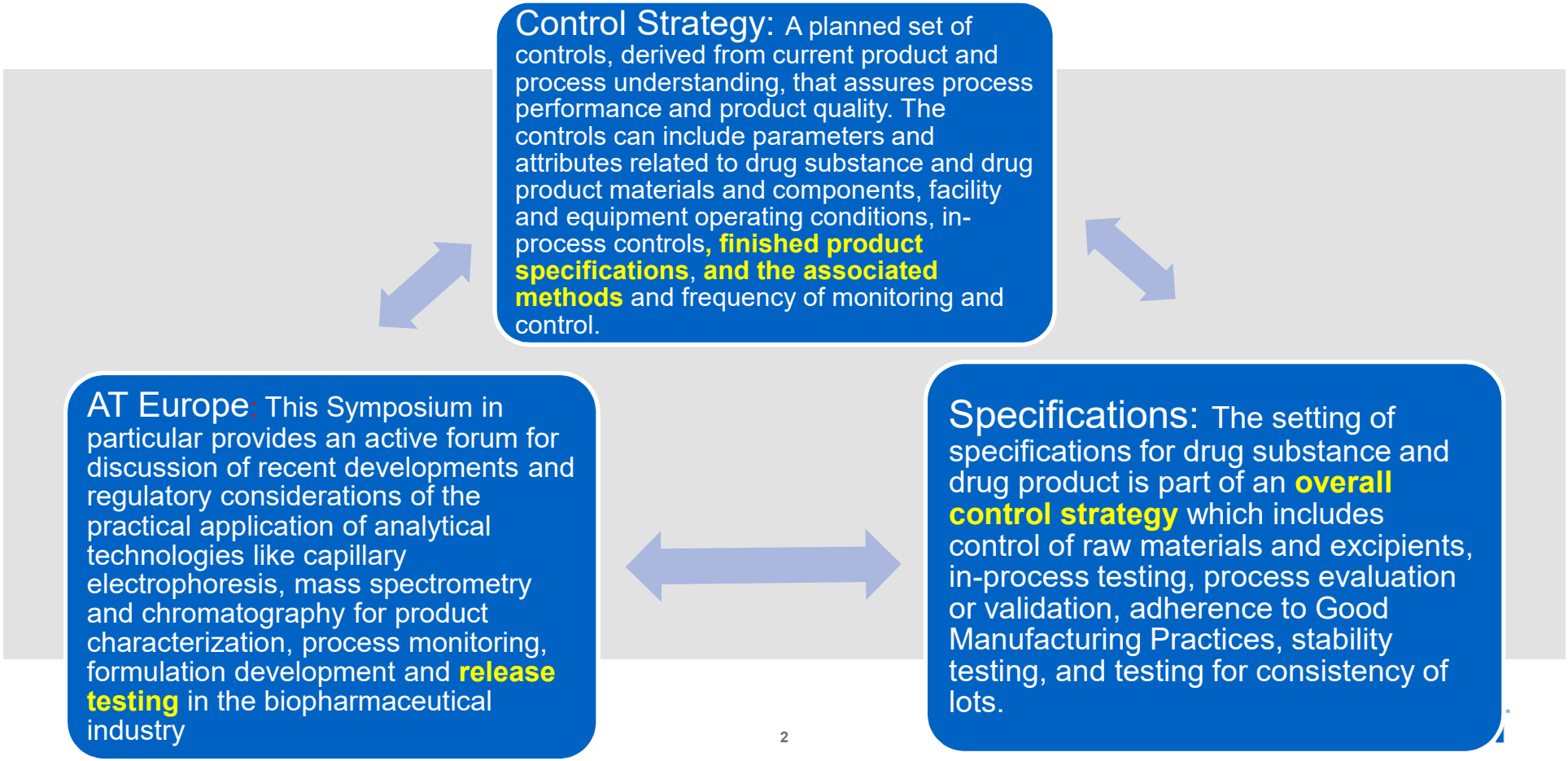
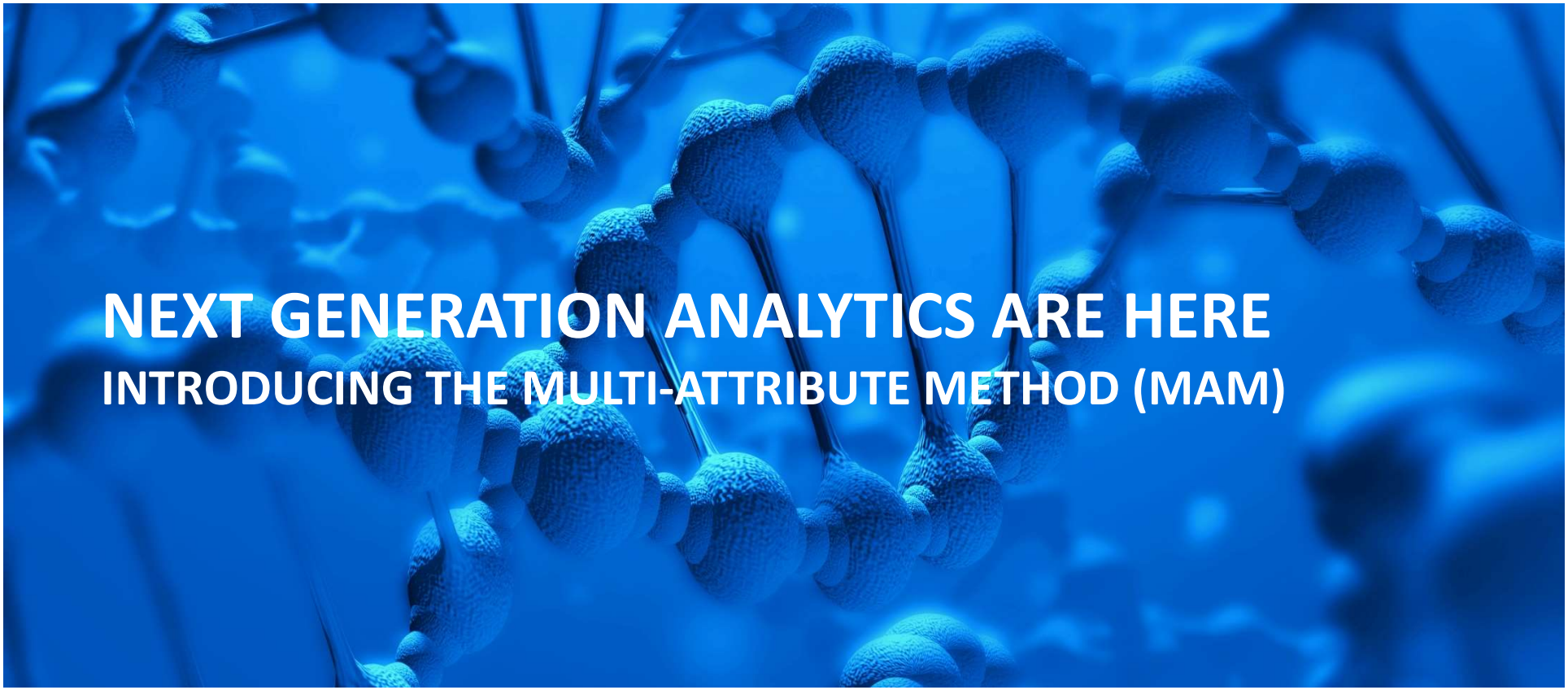
ANALYTICAL TECHNOLOGIES AND CONTROL STRATEGY





NEXT GENERATION ANALYTICS ARE HERE

INTRODUCING THE MULTI-ATTRIBUTE METHOD (MAM)

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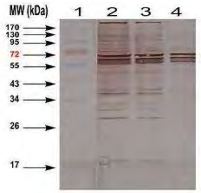
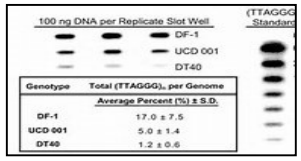
EVOLUTION OF ANALYTICAL TESTING TECHNOLOGIES

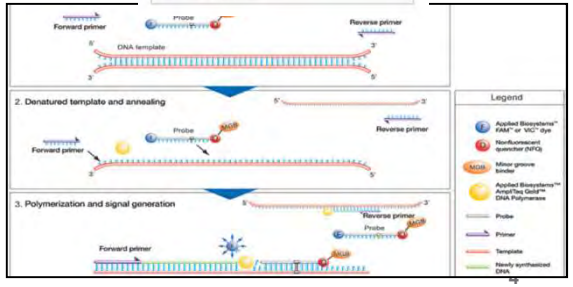
Process Impurity Testing

- **Host cell protein and other process related impurities**



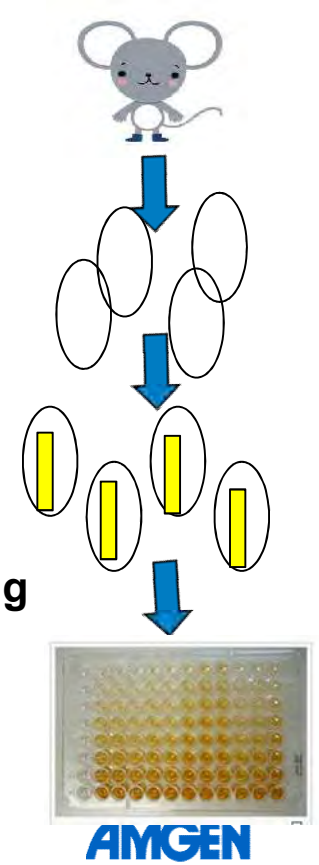
- **DNA**





Potency Testing

- **Whole animal**
- **Primary cultures**
- **Cell lines**
- **Receptor-ligand binding**
- **Antigen binding**



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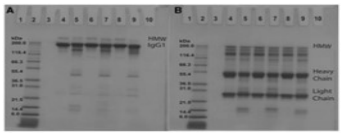
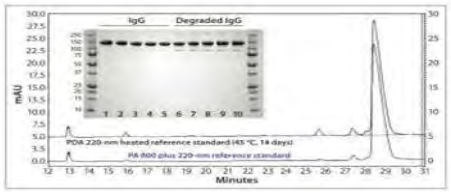
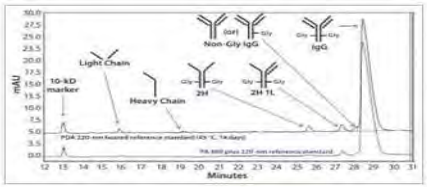
EVOLUTION OF ANALYTICAL TESTING TECHNOLOGIES

Purity Testing (electrophoretic)







Purity Testing (chromatographic)

Separation in ~ hour

Fast Protein Liquid Chromatography - FPLC

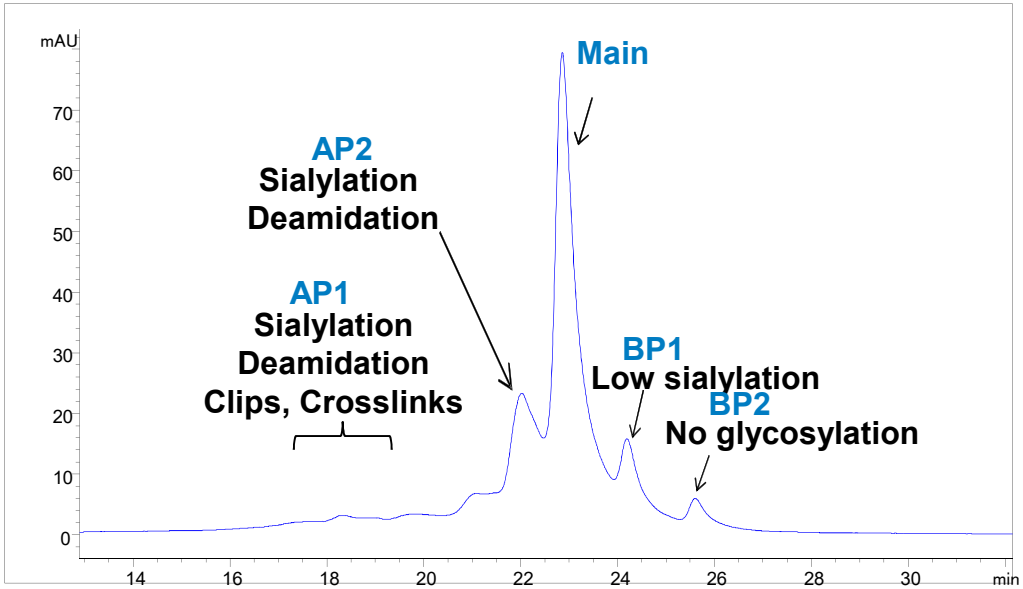
Separation in 15 – 30 min

High Performance Liquid Chromatography - HPLC

Separation in < 5 min

Ultra High Performance Liquid Chromatography - UHPLC

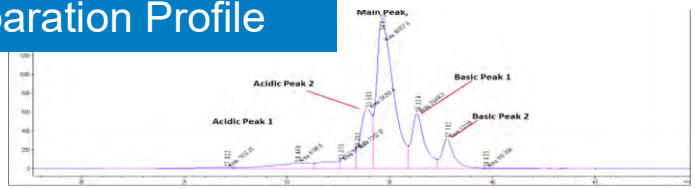
CONVENTIONAL CEX-HPLC ASSAY FOR A MAB – MONITORS PEAKS PROFILE



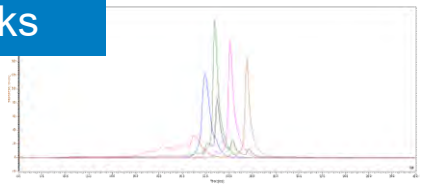
High Resolution Mass Spectrometry is ideal for identification and quantitation of specific attributes

MASS SPECTROMETRY: CHARACTERIZATION TOOL

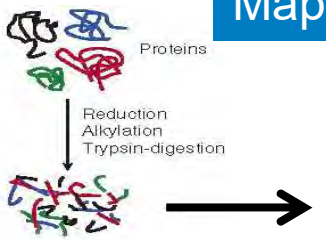
Separation Profile

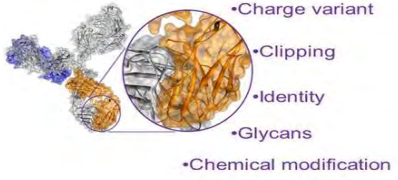


Collect peaks



Mapping species in each peak





Mass Spectrometry allows for identification and quantitation of product attributes

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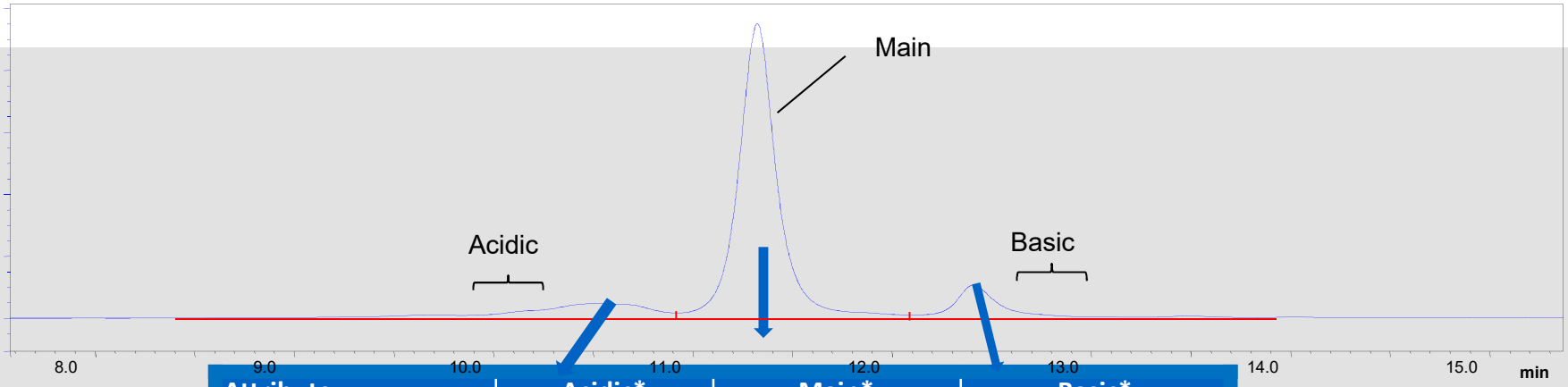
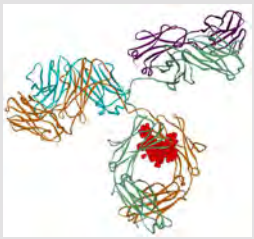
CONVENTIONAL CEX-HPLC ASSAY – MONITORS PEAKS PROFILE





Attribute	Acidic*	Main*	Basic*
HC W^{107} Oxidation	**8.32%**	3.28%	3.86%
LC W^{92} Oxidation	**5.66%**	2.18%	2.90%
LC K^{153} Glycation	**5.54%**	3.86%	3.46%
HC N^{390} Deamidation	**13.18%**	1.84%	1.76%
HC M^{258} Oxidation	**16.22%**	10.26%	12.84%
HC M^{434} Oxidation	10.58%	5.28%	8.70%
LC N-term Q	7.02%	6.30%	**52.18%**
HC C-term K	0.10%	0.10%	**4.68%**
HC N-term pE	6.48%	5.60%	5.84%
HC $D^{276}P$ Cleavage	3.96%	3.86%	3.84%
M5 Glycans	4.66%	5.59%	**8.55%**
A1G0F Glycans	1.88%	2.41%	2.65%
A2G0F Glycans	42.47%	47.73%	41.43%
A2G1F Glycans	39.15%	32.96%	26.96%
A2G2F Glycans	8.69%	6.14%	4.45%

* - content adjusted per molecule

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RECENT PUBLICATION IN AAPS JOURNAL

The AAPS Journal

A View on the Importance of "Multi-Attribute Method" for Measuring Purity of Biopharmaceuticals and Improving Overall Control Strategy

Authors

Richard S. Rogers, Michael Abernathy, Douglas D. Richardson, Jason C. Rouse, Justin D. Sperry, Patrick Swann, Jette Wypych, Christopher Yu, Li Zang, Rohini Deshpande

Abstract

Today, we are experiencing unprecedented growth and innovation within the pharmaceutical industry. Established protein therapeutic modalities, such as recombinant human proteins, monoclonal antibodies (mAbs), and fusion proteins, are being used to treat previously unmet medical needs. Novel therapies such as bispecific T cell engagers (BiTEs), chimeric antigen T cell receptors (CARTs), siRNA, and gene therapies are paving the path towards increasingly personalized medicine. This advancement of new indications and therapeutic modalities is paralleled by development of new analytical technologies and methods that provide enhanced information content in a more efficient manner. Recently, a liquid chromatography-mass spectrometry (LC-MS) multi-attribute method (MAM) has been developed and designed for improved simultaneous detection, identification, quantitation, and quality control (monitoring) of molecular attributes (Rogers et al. MAbs 7(5):881–90, 2015). Based on peptide mapping principles, this powerful tool represents a true advancement in testing methodology that can be utilized not only during product characterization, formulation development, stability testing, and development of the manufacturing process, but also as a platform quality control method in dispositioning clinical materials for both innovative biotherapeutics and biosimilars.

KEY WORDS

biotherapeutic mass spectrometry multi-attribute method quality by design

Published (January 2018, 20:7) in the American Association of Pharmaceutical Scientists
Link directly to AAPS Journal Article:
https://rd.springer.com/article/10.1208/s12248-017-0168-3

Rogers, R.S., Abernathy, M., Richardson, D.D. et al. AAPS J (2018) 20: 7. https://doi.org/10.1208/s12248-017-0168-3

Common issues and concerns around broader implementation of MAM typically fall into four categories: technical, regulatory/compliance, capability as a replacement (instead of an additional) release test, and a diversified regulatory environment.

Industry collaboration outlining the relevance and importance of MAM

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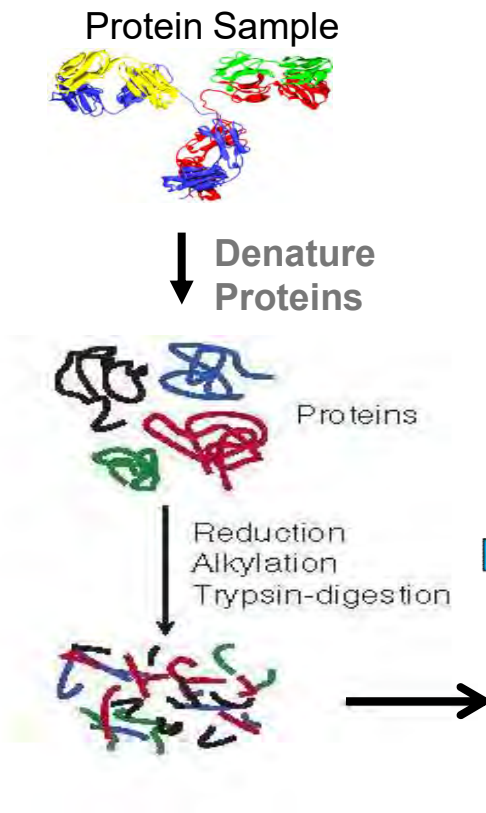
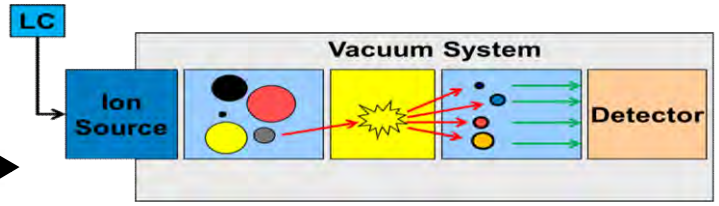
DRIVERS FOR APPLICATION OF MULTI-ATTRIBUTE METHOD

- **Selective and specific monitoring of biologically relevant Product Quality Attributes rather than less specific monitoring by traditional methods (eg. CEX, reduced CE-SDS) better ensures product quality**
- **All covalent PQAs are captured, though not reported, which speeds investigations of process deviations**
- **Reduced number of assays for process development, product disposition and in-process control lowers costs and improves cycle time**
- **Modality independent method speeds process development and embraces the principles of Quality-by-Design (applicable for mAbs, Fc-fusions, BiTE®s, bi-specifics, ADCs)**
- **Smaller footprint due to reduction in number of types of instruments**

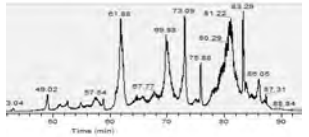
MAM IS A PEPTIDE MAP BASED ON MASS SPECTROMETRY THAT MEASURES SEVERAL ATTRIBUTES IN A SINGLE ASSAY



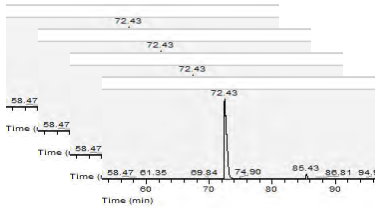
- Denature the sample
- Reduce and alkylate
- Desalt
- Digest with trypsin
- Inject the digest
- LC/MS analysis

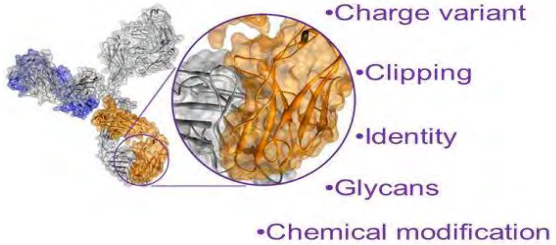


Total Ion Chromatogram

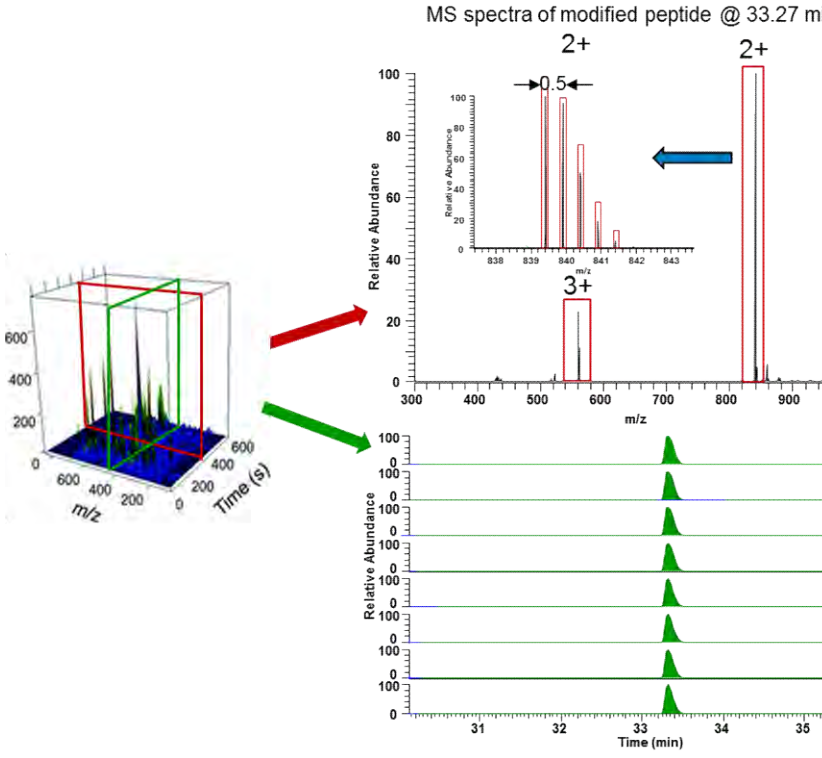


Extracted Ion Chromatograms





PRINCIPLES OF MASS SPECTROMETRY FOR MASS DETERMINATION AND RELATIVE QUANTITATION



Calculate the monoisotopic mass of peptides:

$$\boldsymbol{Monoisotopic\ Mass} = (\boldsymbol{m/z} - \boldsymbol{H^{+}}) \times \boldsymbol{z}$$

Calculate the peak area of modified peptide

Calculate the peak area of unmodified peptide

$$\%\boldsymbol{Mod} = \frac{\boldsymbol{Peak\ Area_{Mod}}}{\boldsymbol{Peak\ Area_{Mod} + Peak\ Area_{Unmod}}}$$

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CRITERIA FOR EVALUATING A PEPTIDE OR ATTRIBUTE USING THE MULTI ATTRIBUTE METHOD

Development

1. Identification of the peptide/attribute is confirmed by MS^2 fragmentation + orthogonal characterization methods (HILIC-MS for glycosylation)
2. The retention time window for the peptide/attribute is defined
3. Set appropriate filters and threshold for new peak in Sieve

Execution

1. **The retention time for the peptide/attribute must be within a set retention time window (determined by characterization of the molecule)**
2. **The experimental mass is less than 5 ppm from the predicted mass**
3. **The experimental isotopic distribution fit to the theoretical must meet pre defined criteria**
4. **Apply filters and Threshold for new peak detection**

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MAM HAS POTENTIAL TO REPLACE SEVERAL METHODS & ASSOCIATED INSTRUMENTS

Current Method	Attribute	Proposed Method
rCE-SDS	Purity - Clips	Multi-Attribute Method (MAM)
CEX-HPLC	Purity – Charge Variants	
Glycan Map	Glycans	
Immunoassay	Identity	

HPLC-FLD (Glycan-map) | **HPLC-UV (CEX-HPLC)** | **CE-UV (rCE-SDS)** | **Platereader (immunoassay)**

MAM replaces four instrument types

UPLC/MS (MAM)

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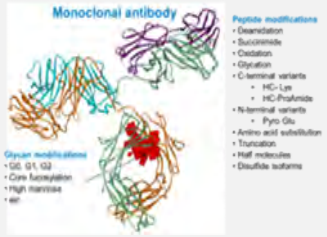
MAM CAN DIRECTLY IDENTIFY AND QUANTIFY PQAS AT AMINO ACID LEVEL WHICH ENABLES AMGEN TO DESIGN RELEVANT QUALITY TARGET PRODUCT PROFILE

Patient Centric Process Development

Target Product Profile

- Indication & use
- Dosage & administration
- Tolerability
- Dosage forms & strength
- Efficacy
- Safety/side effects
- Value & access

Product Quality Attribute Assessments



- CQA identification
- Scoring for potential impact to safety/efficacy

Quality Target Product Profile

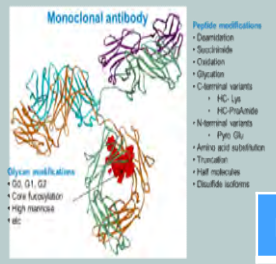
- Critical quality attribute selection
- Attribute range determination
- Designing quality into product during PD

Attribute Measurements

- MAM provides specific attribute measurement
- Allows Amgen to control the levels of individual molecular CQAs

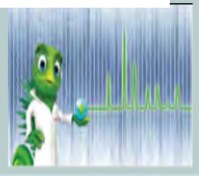
MULTI-ATTRIBUTE-METHOD MEASURES SPECIFIC ATTRIBUTES TO ASSESS 'FIT TO QTPP'

AMG X



Attribute	PQAA
Sialylation	PK – 7
Oxidation	PK – 5
Oxidation	Potency – 5
Deamidation	Potency – 5
Clips	Potency – 5

Multi Attribute Method:



Chromeleon 7.2 SR2
CFR Title 21 Part 11 Compliant Package

Elements of QTPP

Category	Attribute	Target Range	Current Observed Range
Strength	Concentration	126 – 154 mg/mL	131 – 149 mg/mL
Quality	HC Asp Isomerization	≤ 2%	0.1 – 0.5%
	LC Trp Oxidation	≤ 5%	0.1%
	HC Met Oxidation	≤ 5%	0.3 – 0.9%
	HC Met Oxidation	≤ 5%	0.4%
	Met Oxidation	1% – 7%	2.5 – 4.1%
	Met Oxidation	≤ 5%	0.7 – 1.6%
	High Mannose Glycans	2% – 12%	6.2 – 8.5%
	Protein Dimer/Oligomers (SEC HMW)	≤ 1%	0.4 – 0.6%
	Protein Fragmentation (rCE LMW+MMW)	≤ 1%	< 0.6%
	Glycation (LC K)	≤ 5%	0.8 – 1.5%
	Hydroxylysine (HC K)	≤ 2%	< 0.1%
	Hydroxylysine (HC K)	≤ 2%	1.0 – 2.0%
	Osmolality	250 – 350 mOsm/kg	301 – 312 mOsm/kg
	Polysorbate 80	0.005% – 0.015%	0.009 – 0.013%
	pH	4.9 – 5.5	5.1 – 5.2
Safety	Host Cell Protein	≤ 100 ppm	20 – 49 ppm
	Residual Protein A	< 6 ppm	< 1 ppm
	Endotoxin	≤ 0.25 EU/mg	≤ 0.0022 EU/mg
	Bioburden	≤ 10 CFU/10 mL	0

1. Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics
   Rogers RS, Nightlinger NS, Livingston B, Campbell P, Bailey R, Balland A. *MAbs*. 2015; 7(5): 881-890
2. An improved trypsin digestion method minimizes digestion-induced modifications on proteins
   Ren D, Pipes GD, Liu D, Shih LY, Nichols AC, Treuheit MJ, Brems DN, Bondarenko PV. *Anal Biochem*. 2009; 392(1): 12-21

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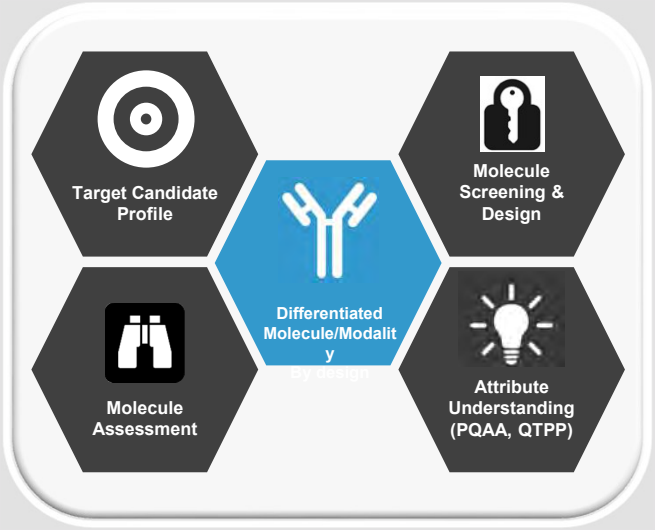
ATTRIBUTE-BASED STRATEGY WILL ENABLE MOLECULE DIFFERENTIATION TO MEET PATIENT NEEDS



- **Apply 'Quality Target Product Profile' to meet patient needs defined within Target Product Profile**
- **Deliver attribute understanding, methods to test and control them, and ensure supply for patients**
- **Advance new attribute technologies for specific, fast and multi-attribute methods**

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PARTNERING AROUND THE WORLD TO ADVANCE NOVEL TECHNOLOGIES FOR ATTRIBUTE MONITORING AND CONTROL

Sensor technologies

- Proteins and raw material ID

Multi-attribute Method

- Measures multiple attributes
- DS/DP release/stability method
- Replaces 3-6 methods
- Molecule characterization

NMR

- Solid state NMR: Monitor potential for conversion to amorphous phase in tablets
- 1D Profile NMR / 2D NMR technologies for comparability & biosimilarity

Automated Liquid Handlers

TECAN

- ELISAs (DNA, HCP, ProA ELISA)
- Auto Sample Dilutions

ECHO

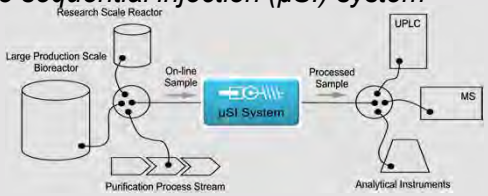
- Acoustic droplet ejection technology for improved assay precision and throughput

Process Analytical Technologies

Chemical Process Interrogation Platforms

In-situ probes:
- particle size measurement - PVM/FBRM
- on-line reaction monitoring - ReactIR

Micro sequential injection (µSI) system



- Flow-based chemistry through novel physical design and software coding.
- Connectivity between sample source and online real-time analytics.

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REGULATORY CONSIDERATIONS

INITIAL REGULATORY FILING STRATEGY

- Implementation of MAM requires well defined regulatory strategy
- Amgen applied MAM principles to regulatory filings using a stepwise and phase appropriate risk-based approach
  - For early stage clinical products (First in Human)
    - Introduce MAM as characterization method as part of S.3.1 Elucidation of Structure
    - Based on successful acceptance, apply MAM as choice method for product disposition (S.4 Control of Drug Substance)
  - Late stage pivotal clinical products (Phase 3 to Commercial)
    - Based on continued success from early stage programs, include MAM as choice method for product disposition on specifications (DS/DP and Stability)initial
  - Life-cycle products
    - Based on late-stage pivotal acceptance replace conventional testing methods with MAM
- Using MAM on specifications for product disposition
  - Types of modifications reported: deamidations, oxidations, glycations, glycoslylations, sialylations, clips, etc.
  - Numerical acceptance criteria would be determined as experience and data are gathered

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MAM IMPLEMENTATION FOR PIPELINE MOLECULES

Regulatory Feedback

- Acceptance of MAM as characterization method
- Acceptance of MAM as release method for ID & Glycan
- Requested additional information on comparability vs conventional methods
- Requested additional information on qualification, robustness, and precision of MAM
- 5/1/2017- FDA response received. Requested additional information on MAM technical capability.
- 7/12/2017 – FDA teleconference. FDA very engaged in moving MAM forward as replacement QC method.

Q4 2014 - Q2 2015	Q3 2015	Q3 2016	Q1 2017	Q2 2017	Q2 2017	Q4 2017	Q1 2018
Characterization (IND S.3.1)	GMP Release (IND S.3.1, S.4.4)	Type C Meeting with US FDA	MAM INDa submitted	EU CTA (FR & BG)	GMP Release	Meeting Engagement with JP PMDA	Meeting Engagement with CFDA

Clinical CTAs/INDs

Product 1 Product 2 Product 3 Product 4	Product 1	Product 1	Product 1	Product 1	Product 5 Product 6 Product 7 Product 8 Product 9

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REGULATORY FEEDBACK – WCBP 2018 FDA COMMENTS

- **Data on method performance is not normally submitted in the initial IND but "qualification data" are expected to be available, if requested.**
- **Early in development methods should provide meaningful results and should have data supporting method capability including specificity, linearity, accuracy, precision, robustness and stability.**
- **If the analytical method is novel, a summary of method performance is expected.**
- **Full method validation is not expected during development**

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REGULATORY FEEDBACK – WCBP 2018 FDA COMMENTS

- Some sample preparation steps can alter specific QAs.
- Bottom up approaches may not be/are not sufficient.
- Are you analyzing the correct attributes?
- You've identified and quantified specific PTMs and sequence variants, but do you know if they are evenly distributed across molecules or only on 10% of the population?

REGULATORY FEEDBACK – WCBP 2018 FDA COMMENTS

- If the PTM has the potential to affect potency or activity, does knowing the overall level tell you what you need to know? For example, if CDRs of a mAb may be prone to 2 PTMs, is one PTM sufficient to reduce potency or would both PTMs be needed, on one or both halves of the molecule ?
- May not be able to tell you if there was an overall shift in the PI of the product, which could affect PK of sc administration

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REGULATORY FEEDBACK – WCBP 2018 FDA COMMENTS

- However, may be better for setting a spec around a specific PTM with a known impact, rather than setting a spec on an acidic or basic peak.
- If you want to use MS for in-process testing instead of release testing, are you using it in the correct place during manufacture? Can the attributes you are assessing be affected by steps downstream of where you are testing?
- Have you performed an adequate risk assessment of the testing strategy on potency, PK, safety and immunogenicity? Does the MAM give you the information you/we need in order to make appropriate decisions?

LIFECYCLE MANAGEMENT CONSIDERATIONS

Analytical Procedures and Methods Validation for Drugs and Biologics

- If a risk-based evaluation or other drivers lead to changes in an analytical procedure or replacement with a new method or if the procedure is transferred to a new testing site; revalidation, a new validation exercise, an analytical method comparability study, or a combination of these exercises should be considered.

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LIFECYCLE MANAGEMENT CONSIDERATIONS

Analytical Procedures and Methods Validation for Drugs and Biologics

- …to ensure that the analytical procedure maintains its critical performance characteristics (e.g., specificity, precision, accuracy). The degree of revalidation depends on the nature of the change.

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LIFECYCLE MANAGEMENT CONSIDERATIONS

TECHNICAL AND REGULATORY CONSIDERATIONS FOR PHARMACEUTICAL PRODUCT LIFECYCLE MANAGEMENT

Q12

- When there is an increased understanding of the relationship between method parameters and method performance defined by a systematic development approach including robustness studies, ECs are focused on method-specific performance criteria (e.g., specificity, accuracy, precision) rather than a detailed description of the analytical procedure

AMGEN

LIFECYCLE MANAGEMENT CONSIDERATIONS

TECHNICAL AND REGULATORY CONSIDERATIONS FOR PHARMACEUTICAL PRODUCT LIFECYCLE MANAGEMENT

Q12

- Structured Approach to Analytical Procedure Changes**
  - High level description remains unchanged
  - Validation results demonstrate revised method is equivalent or better
  - Test results of revised method provide same quality decision
  - Comparative test results meet criteria of validation protocol
  - System suitability established for revised method

**N/A if spec will change, current spec does not reflect complexity, method uses bio reagent, etc.

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SUMMARY

1. External and internal factors are driving changes in biologics production which require new operational capabilities and flexible manufacturing operations
2. Advancements in attribute based testing paradigms can now be leveraged
3. Amgen will continue to advance progressive Next Generation Advancements to further optimize innovative drug development to deliver for patients around the world

ACKNOWLEDGEMENTS

- Jette Wypych
- Mike Abernathy
- Rohini Deshpande
- Izydor Apostol
- Da Ren
- Sabrina Benchaar
- Quanzhou Luo
- Zhongqi Zhang
- Cenk Undey
- Tura Camilli
- Tamer Eris
- Alicia Zeng
- Gang Xue
- Richard Wu
- Darrin Cowley
- Barry Cherney
- Chetan Goudar
- Jim Thomas
- Stephan RÖenninger

THANK YOU!