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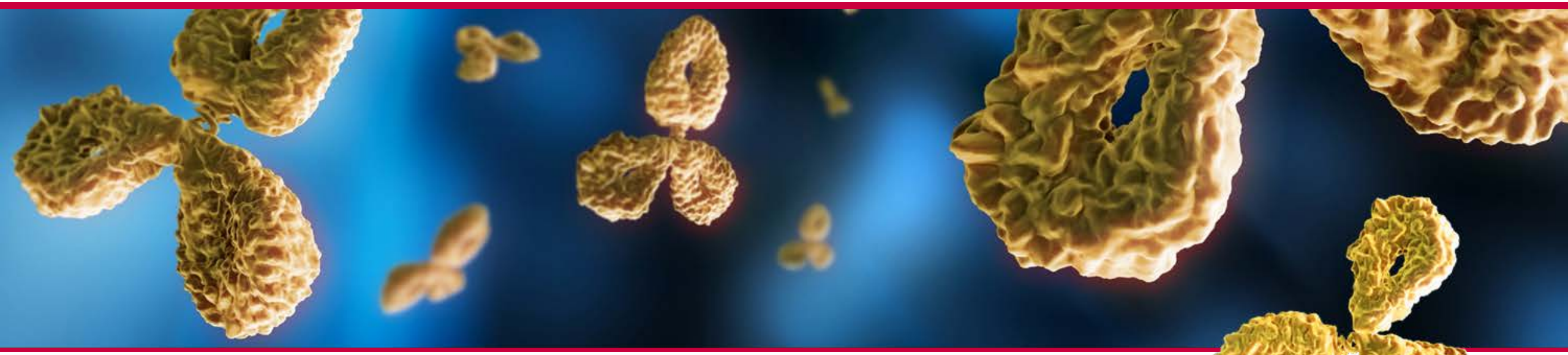
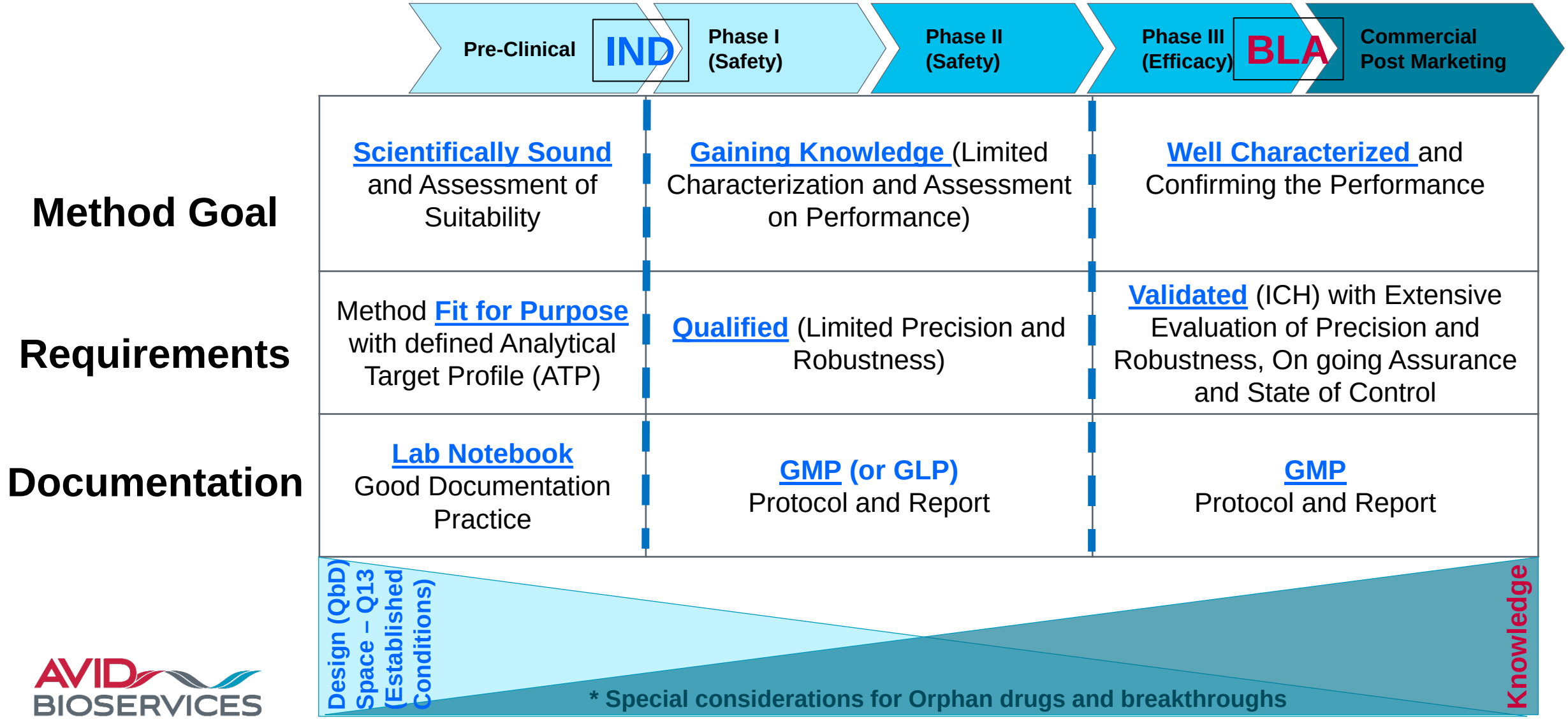


Early Stage Analytical Considerations for Late Stage Success

Arugadoss Devakumar, Ph.D.
Director, Analytical Development
Avid Bioservices Inc.

Tuesday, December 3rd , 2019

Integration of Analytical Release Methods into Product Life Cycle



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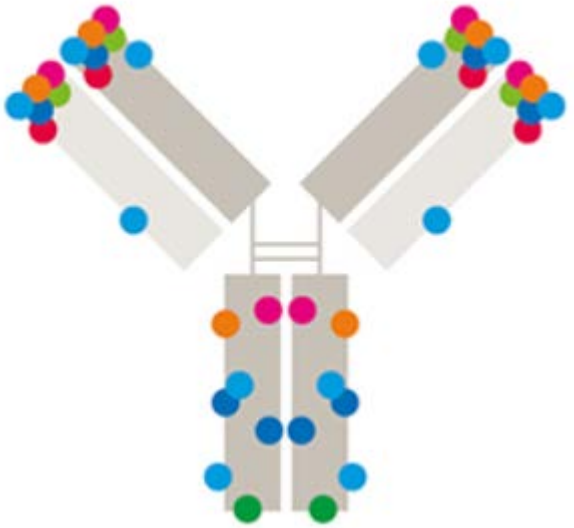
Know Your Protein and Hot Spots!



- Deamidation (Asn – Asp)
- Isomerization (Asp – Iso Asp)
- Oxidation (Met)
- N-glycosylation
- Free Thiol (-SH)
- Pyro-Glutamate
- C-terminal Lysine

Risk Assessment - “must” have and “nice” to have methods

Quality Attribute
(Choosing Right Engine)

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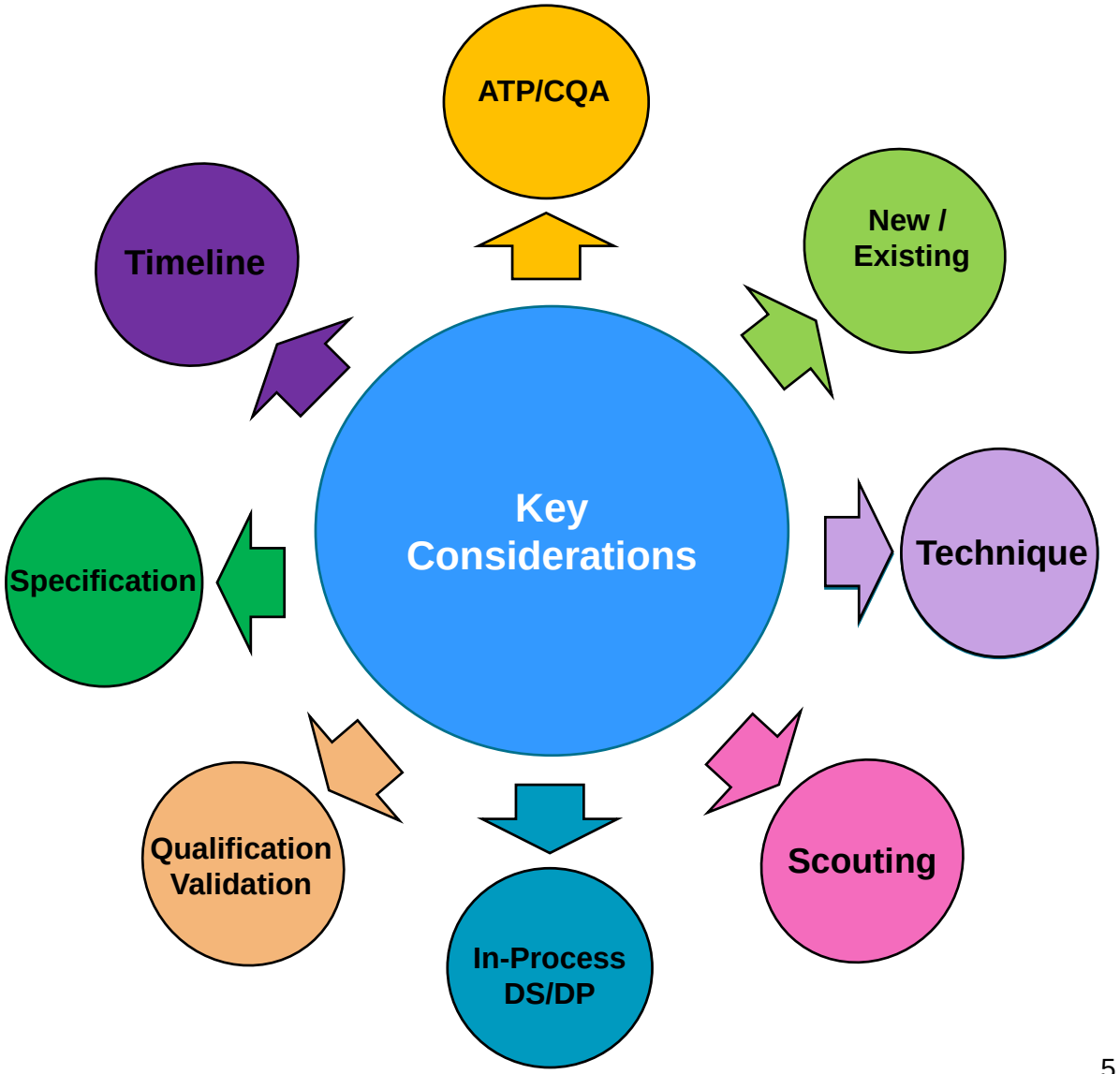
Method Development Elements

Stage	Product QbD	Analytical QbD
1	Define Quality Target Product Profile (QTPP)	Define Analytical Target Profile (ATP)
2	Product Critical Quality Attributes (QA)	Method Critical Quality Attributes (QA)
3	Risk Assessment	Method Risk Assessment
4	Design Space (or) Established Conditions (Q13)	Method Operable Region (Design Space)
5	Control Strategy	Control Strategy (System Suit, Acceptance Criteria)
6	Life Cycle Management	Life Cycle Management

Ramalingam et al Int J Anal chem, 2015



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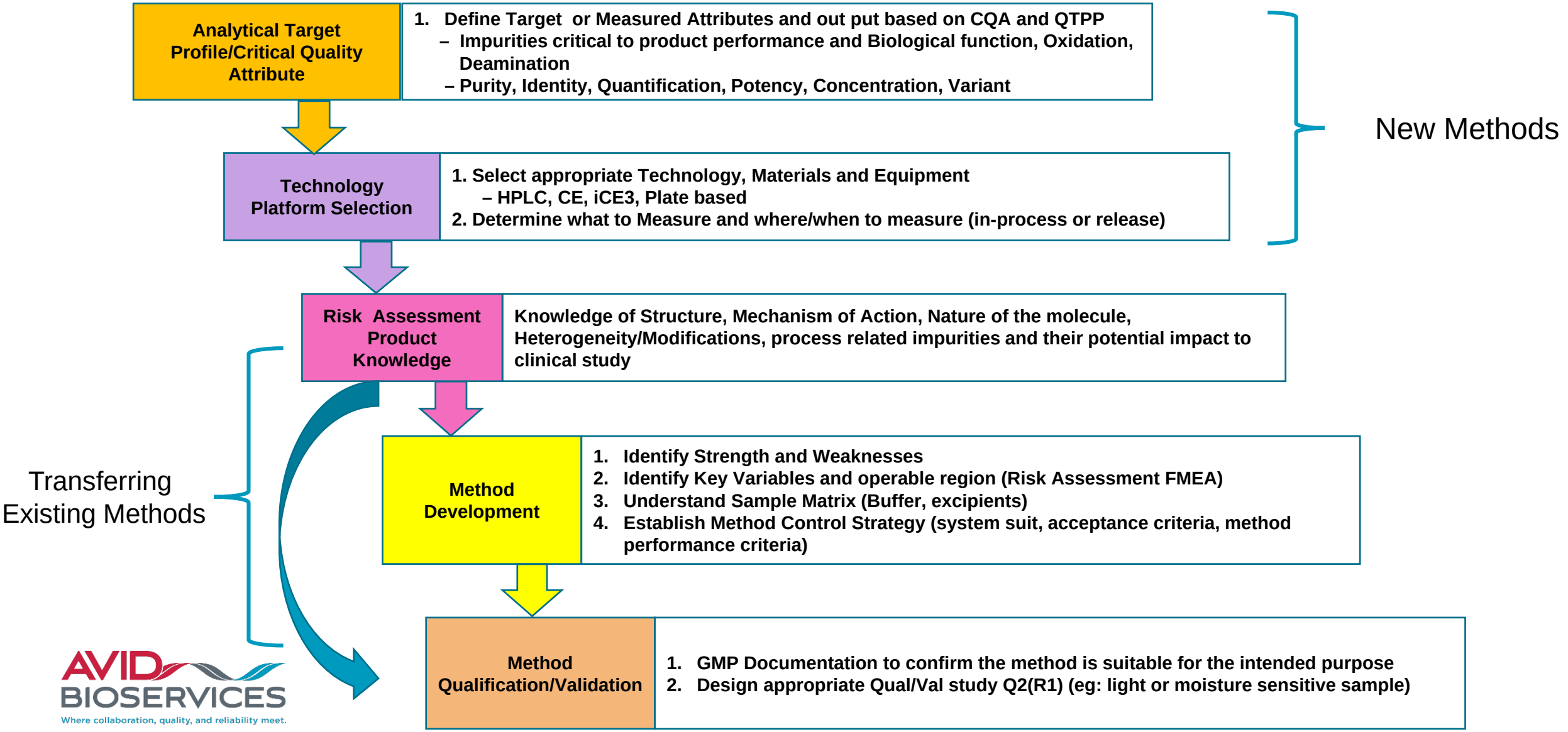
Method Dev/Validation Break Down



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Considerations for Method Development / Validation / Transfer

Running Check List

Sample Prep Section

- ❑ Sample is in What Buffer
- ❑ Any filtration/purification before analysis
  - Evaluate sample loss limit/range
- ❑ What Buffer to be used for Prep/dilution, pH (how many steps/volumes-error rate)
- ❑ Sample Storage container suitability
- ❑ Use of secondary tools required
  - syringe to take sample of of vial
  - dropper/tips/needles
- ❑ Any Heating or Cooling of sample
  - how well they are sealed, tolerance limit
  - water bath or hot plate (thermocycler)
- ❑ Any chemical rxn reduction, alkylation etc

HPLC Method Section

- ❑ Define/Understand the **Scope and Goal** of the method
  - ID/Purity/Quant
  - **CQA and what stability indicating parameter** this method targets
  - Method is developed to capture/cover what? (type of impurities, N-1, N+1 and what are the limits)
- ❑ Types of solvents/Buffers for mobile phase, pH
  - % or ratio of mixing and its error limits
  - type of water (Milli-Q or DI or Inj. Grade)
  - stepwise guidelines for order of mixing
  - evaluate volume error/miscibility issues
  - minimize salt as much as possible
  - filtration required? Temp (don't say RT, give 25 ± 1 or 2 C
- ❑ System/Column clean up before use to avoid carryover
  - make sure seal wash set up in method to extent lifetime
  - define carryover limits

Data Processing Section

- ❑ Type of integration
  - baseline integration preferred
- ❑ Baseline drift level
- ❑ Resolution criteria
- ❑ System suit sample selection and how SS pass/fail correlated to sample pass/fail, which inturn related to CQA of the method
- ❑ Provide data in old and new column to show where it breaks, how it is taken in to system suit pass/fail
- ❑ Accepted level of baseline noise/drift

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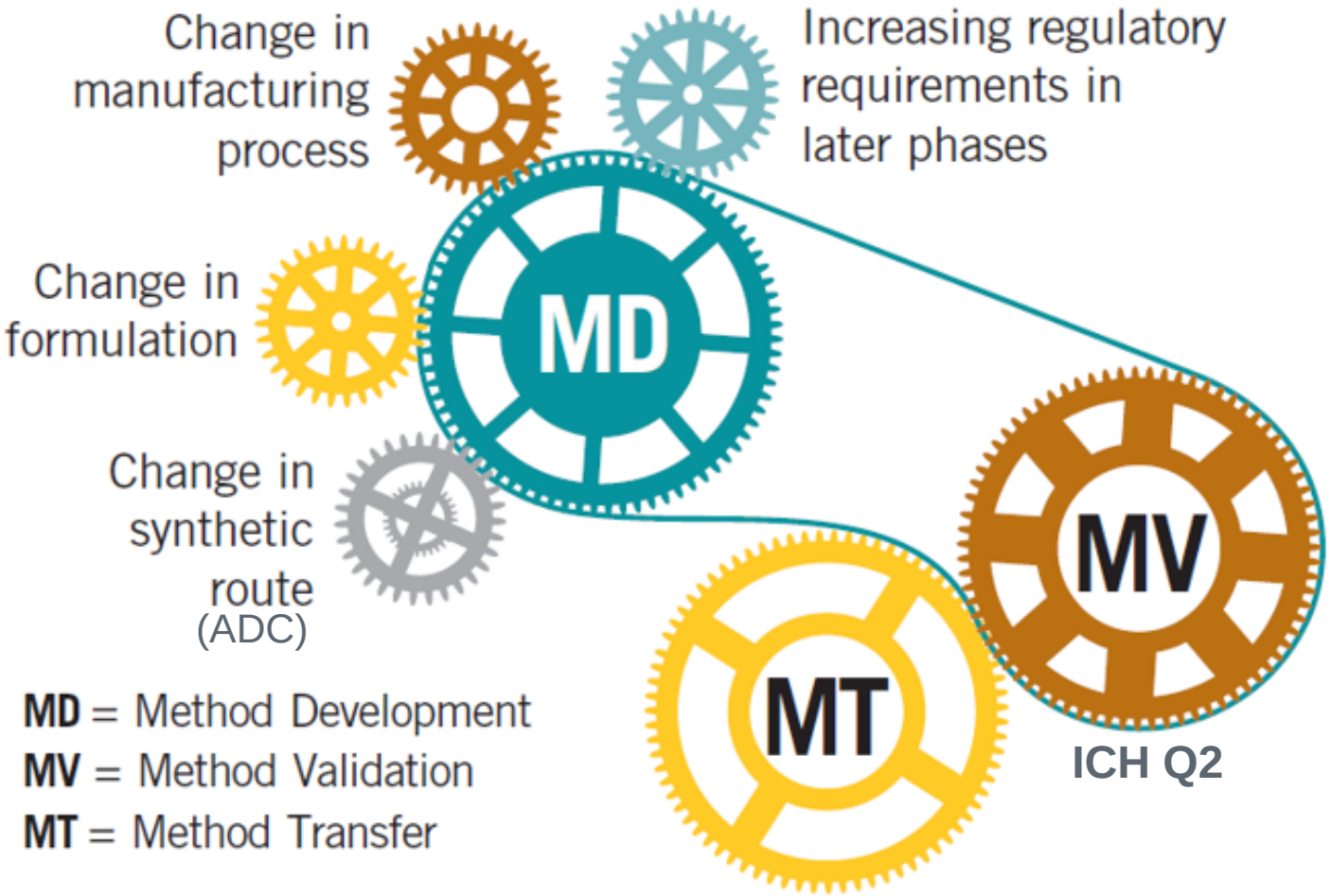
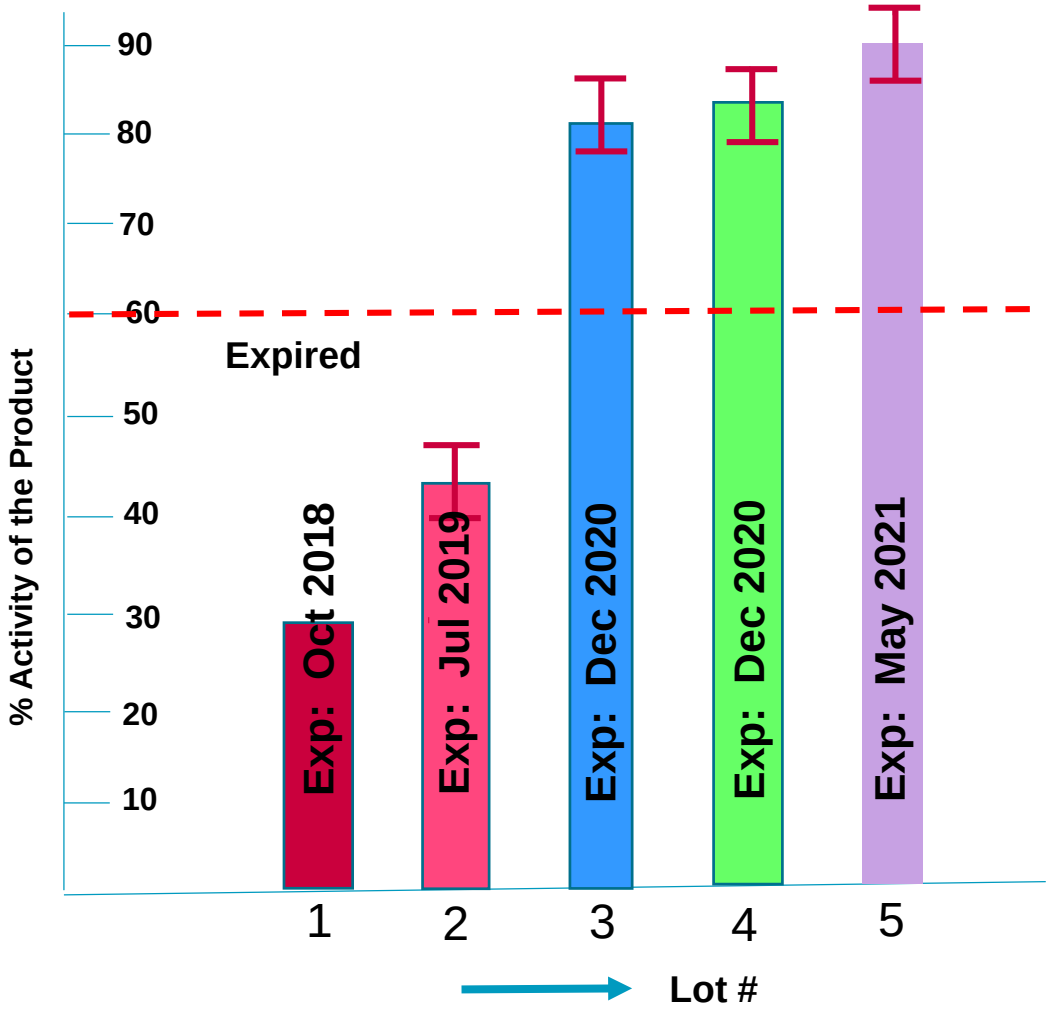
Method Development Life Cycle



Robert W, Lee and Lourie Goldman; Life Science Connect

Stability Indicating Capability of an Analytical Method (Enzyme Activity Assay)



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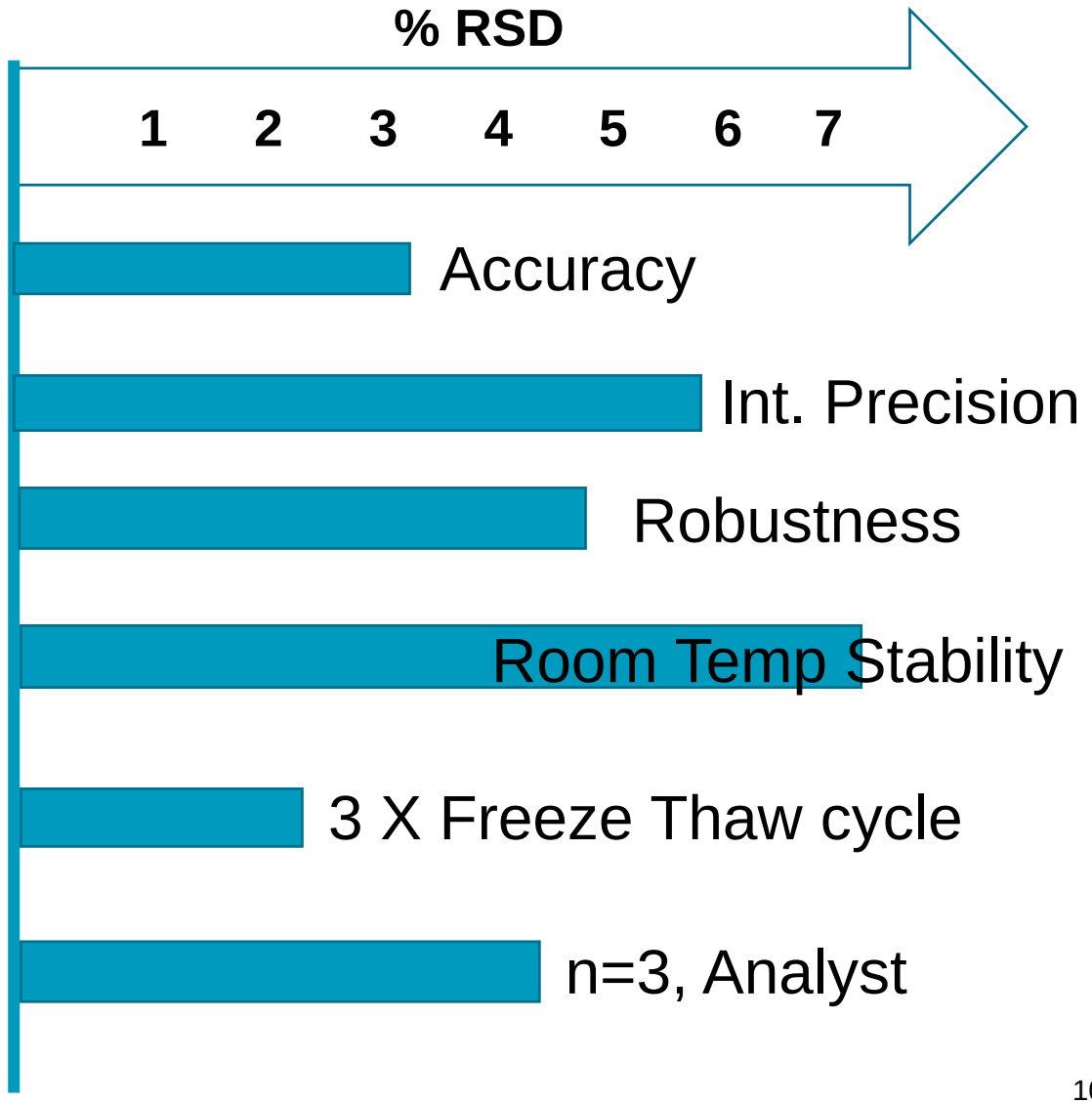
Qualification and Validation of Analytical Methods

- Qualification/validation demonstrates that the analytical procedure is *suitable for its intended use*.
- Defining the Critical Quality Attribute and Stability-indicating Capability
- Can we trust past and future data (Reliable, Consistent)



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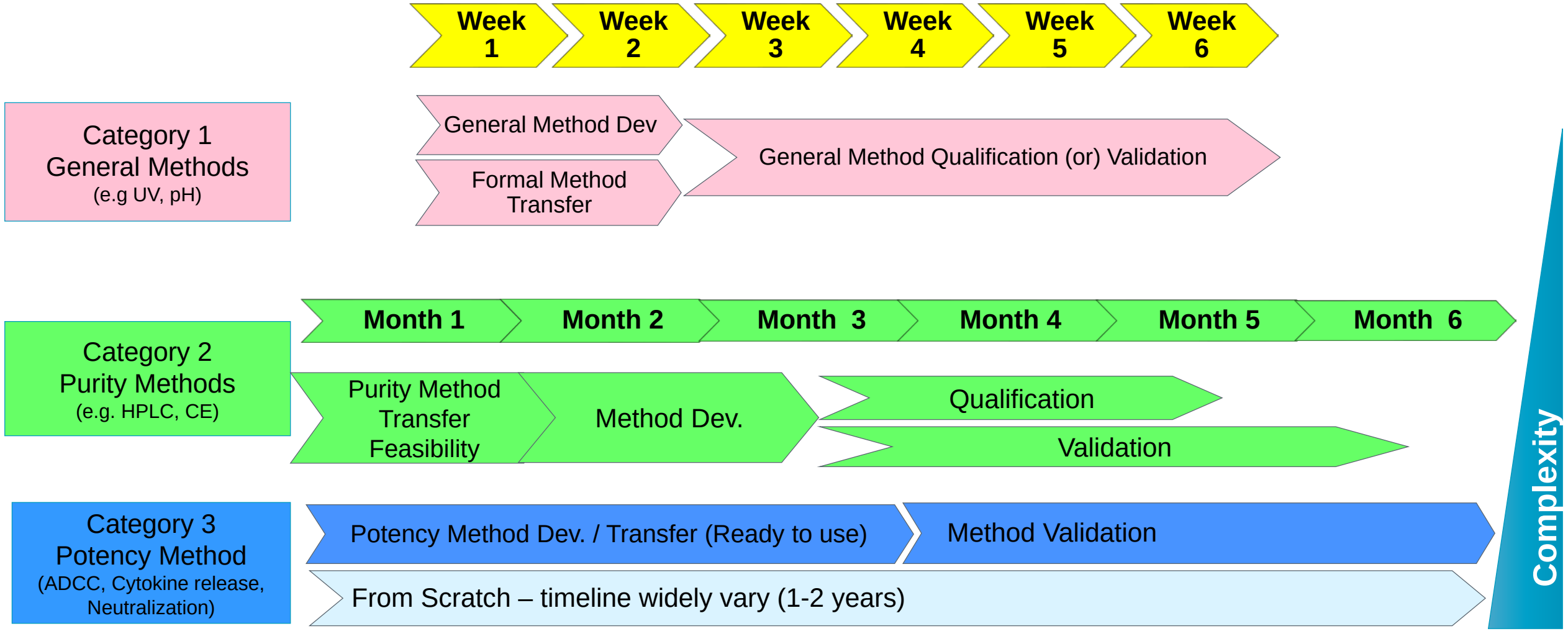
Typical Analytical Method Readiness Timeline



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Product Analytical Characterization

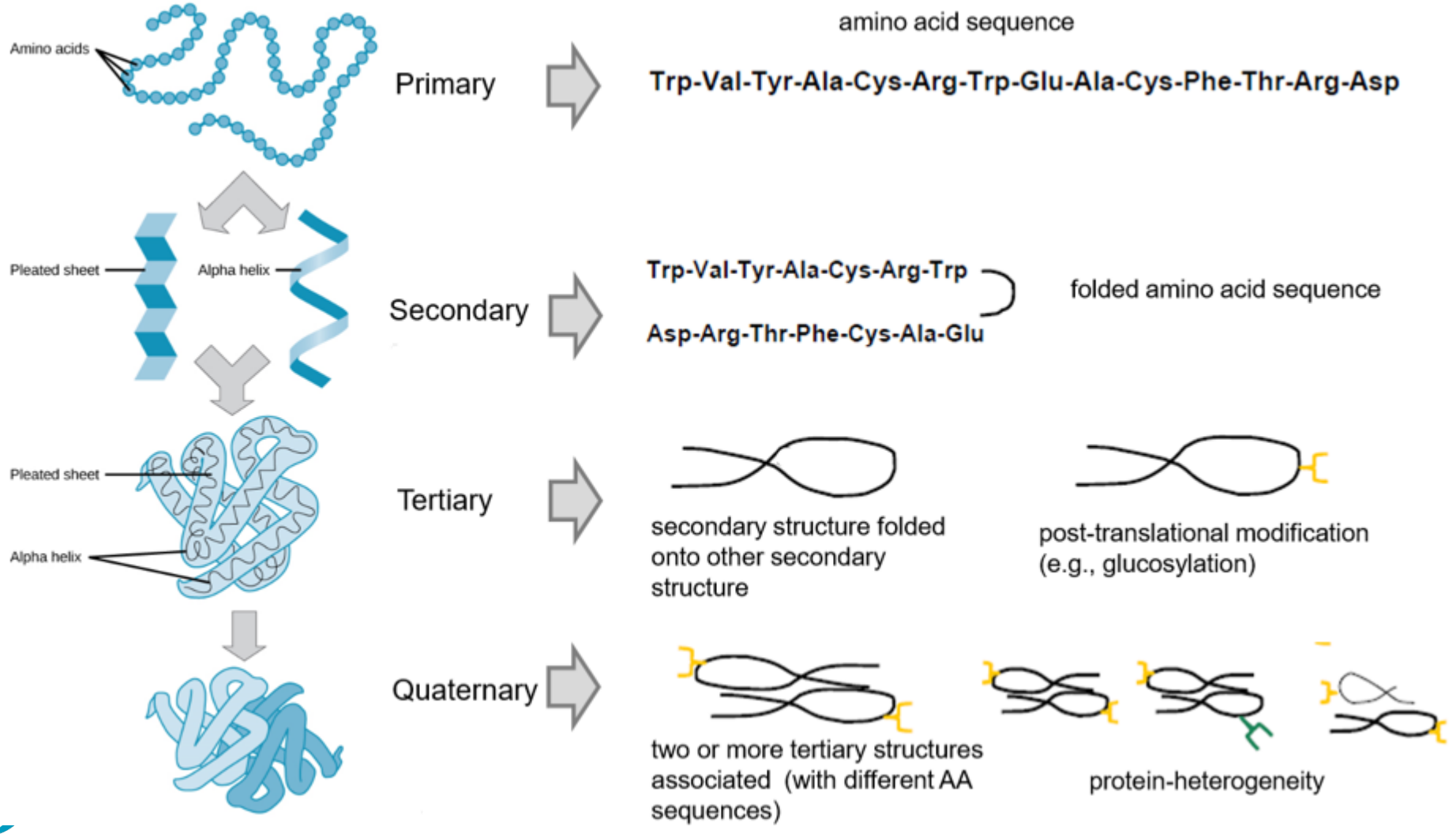

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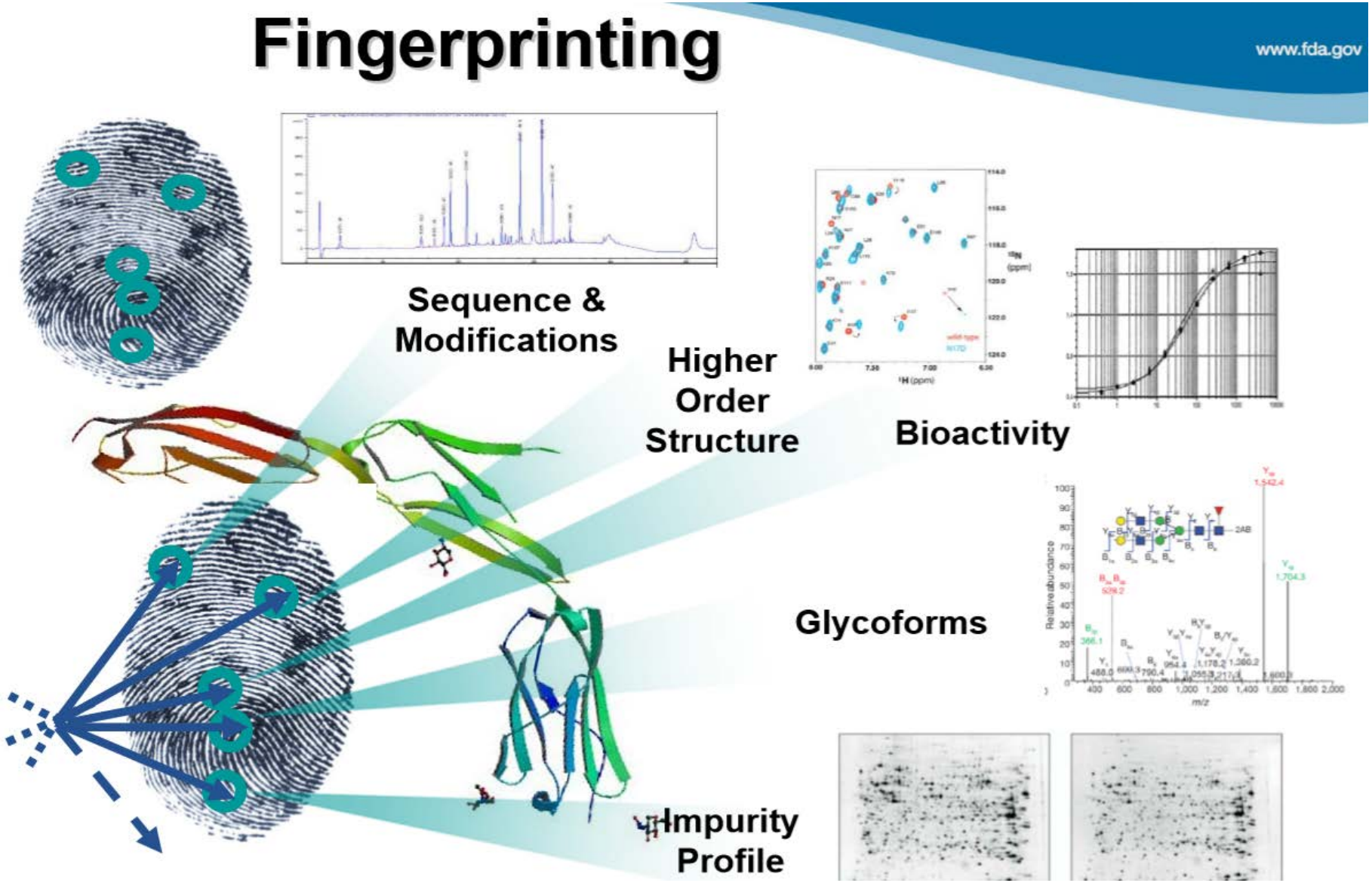
Image adapted from National Human Genome Research Institute

An Exercise in Pattern Recognition



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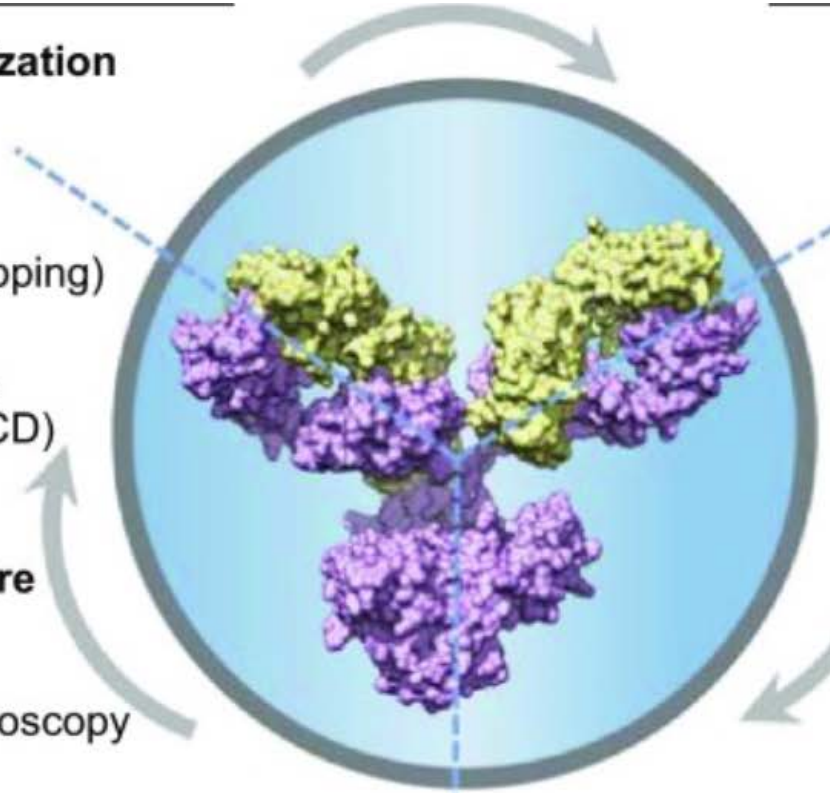
Overview of Molecular Structural Analysis and Functional Assessment

Molecular characterization

- **Primary structure**
  - ESI-MS
  - LC-MS (Peptide mapping)
  - CE-SDS
- **Secondary structure**
  - Circular dichroism (CD)
  - Fourier transformed infrared (FT-IR)
- **Higher order structure**
  - HDX-MS
  - XRD
  - Fluorescence spectroscopy
- **Glycosylation**
  - CE-LIF
  - HPLC (IEX, HILIC etc.)
  - LC-MS (Peptide mapping)
- etc.



Functional assessment

- **Equilibrium dissociation constants**
  - Biacore
  - Fluorescence ELISA (FL-ELISA)
  - Kinetic exclusion assay (KinExA)
- **Ligand binding assay**
  - *In vitro* potency assay (IVRP)
  - Competition ELISA
  - Biacore
- **Cell-based assay**

Analysis of effector functions

- **ADCC, ADCP & CDC assay**

Protein Cell 2018, 9(1):74–85

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Integration of Analytical Characterization Methods into Product Life Cycle

Pre-Clinical → **IND** → **Phase I (Safety)** → **Phase II (Safety)** → **Phase III (Efficacy)** → **BLA** → **Commercial Post Marketing**

- ❑ Tox Study
- ❑ Limited Structural Characterization
  Mainly Primary Structure
  - Sequence info (Peptide Map)
  - Sequence Variants
  - Amino acid Mis-incorporation
  - Glycan Profile
- ❑ Biological Characterization (Immuno assay, Binding assay)
- ❑ Lab-scale Viral Clearance
- ❑ Limited R&D lot stability
- ❑ Limited Forced Degradation Study
- ❑ Accelerated Stability

- ❑ In-depth Structural Characterization
- ❑ 1° Structure
  - Sequence info-Peptide Map
  - Sequence Variants
  - Amino acid Miss-incorporation
  - Glycan Profile
- ❑ 2° Structure
  - Circular Dichroism (UV Far/Near, FL)
  - Binding Assays, BLI, FcɣR
  - DSC
- ❑ Continue Stability Studies
- ❑ Extensive Viral Clearance
- ❑ Manufacturing Scale up
- ❑ Selective Leachables/Extractables
- ❑ In-depth Forced Degradation Study

- ❑ Extended Structural Characterization
- ❑ 1° Structure
  - Sequence info-Peptide Map
  - Sequence Variants
  - Amino acid Miss-incorporation
  - Glycan Profile
- ❑ 2°, 3° and 4° Structures
  - Circular Dichroism (UV Far/Near, FL)
  - Binding Assays, BLI, FcɣR, SPR
  - DSC
  - HDX, 2 or 3D NMR, SAR
- ❑ Full Scale Leachables/Extractables
- ❑ Confirmatory Stability
- ❑ Final Specification Setting

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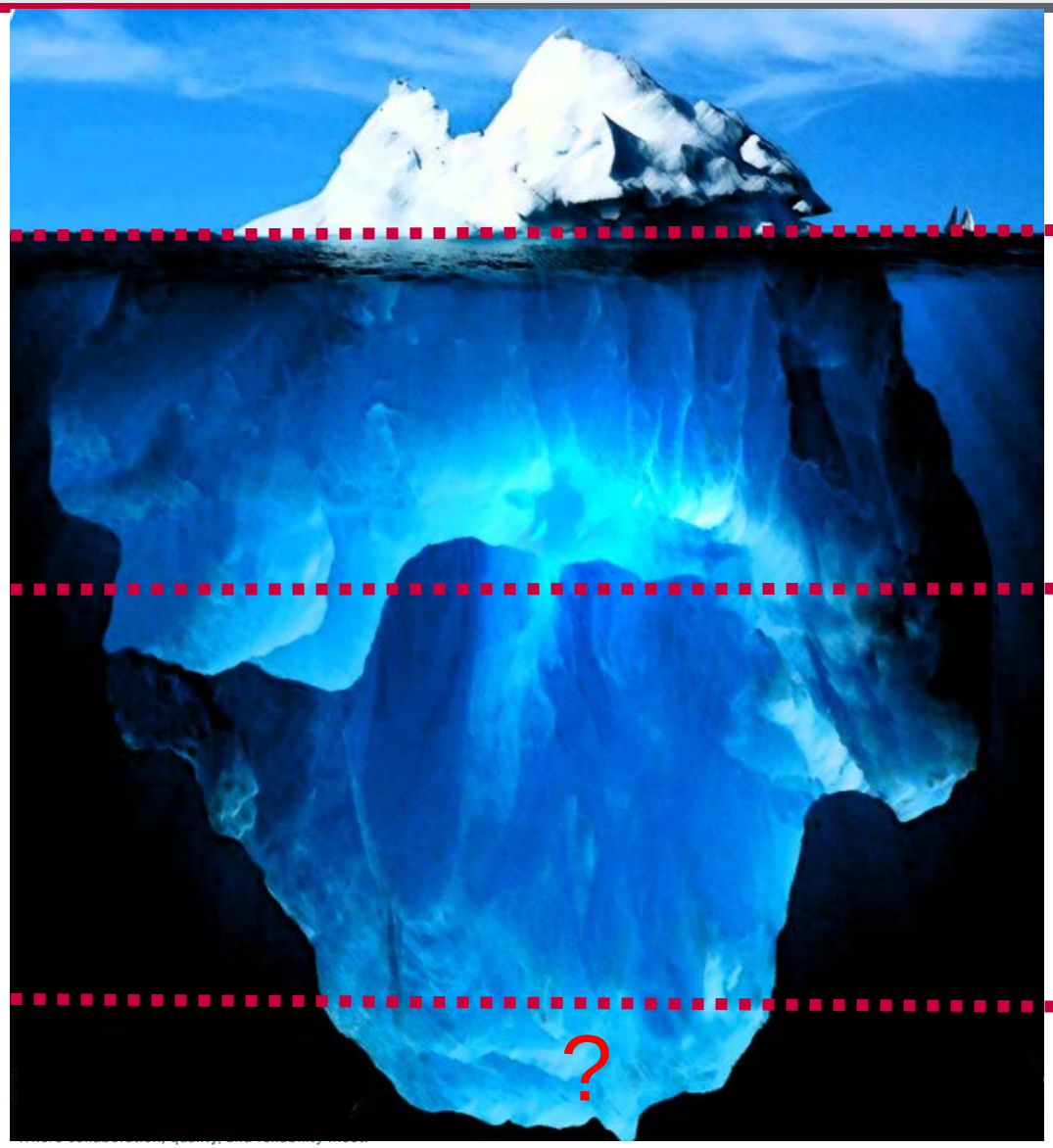
* Special considerations for Orphan drugs and breakthroughs

Quality Profile



Release Tests
(Specifications)

Extended Characterization

- **Process**
- **Product**

Process Control

- **Procedures**
- **Materials**
- **In-process Testing**
- **Monitoring**
- **Validation**

Unknown (Learned overtime)
Update Control Strategy

Analytical Method Transfer Case Study 1

Drug Product

Pen

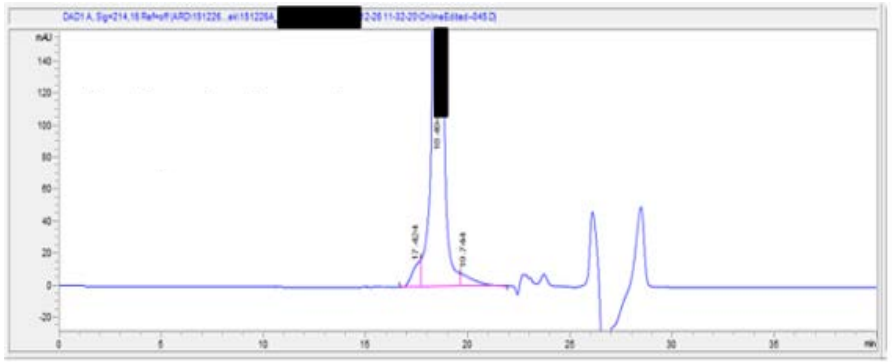
Cartridge

Sterile NORM-JECT®
used to transfer sample
in to HPLC vial

HSW® Norm-Ject® Sterile Luer-Slip ...

Sterile NORM-JECT®
Sample transfer syringe

Size Exclusion
Chromatography
(SEC-HPLC)



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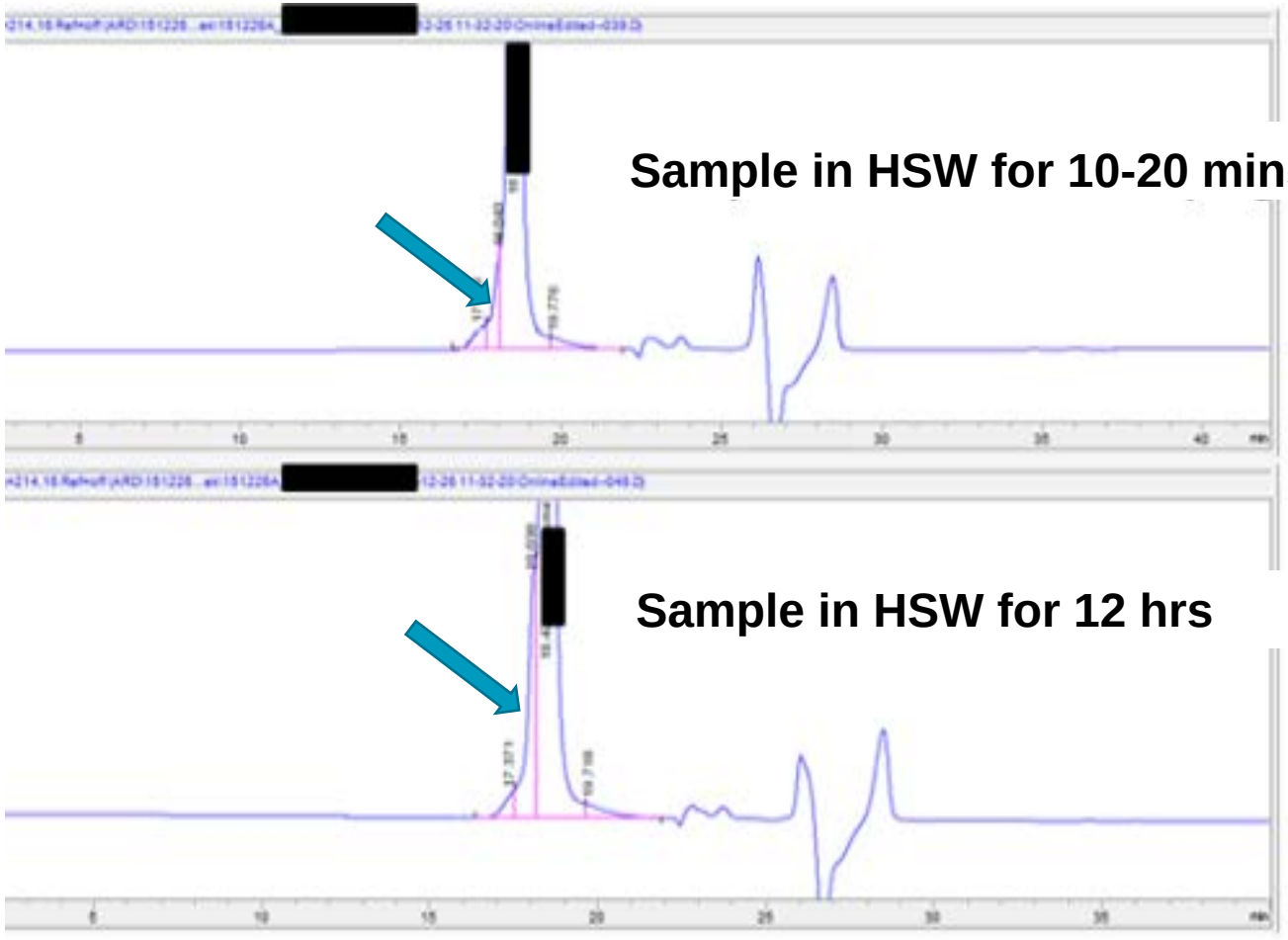
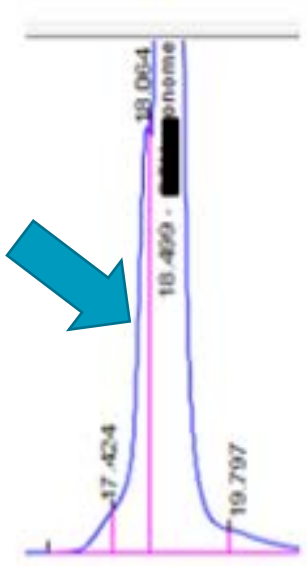
GMP Method Transfer Case Study 1

HSW® Norm-Ject® Sterile Luer-Slip ...





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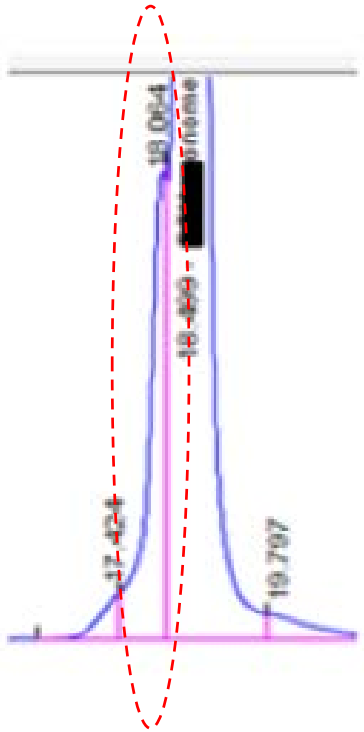
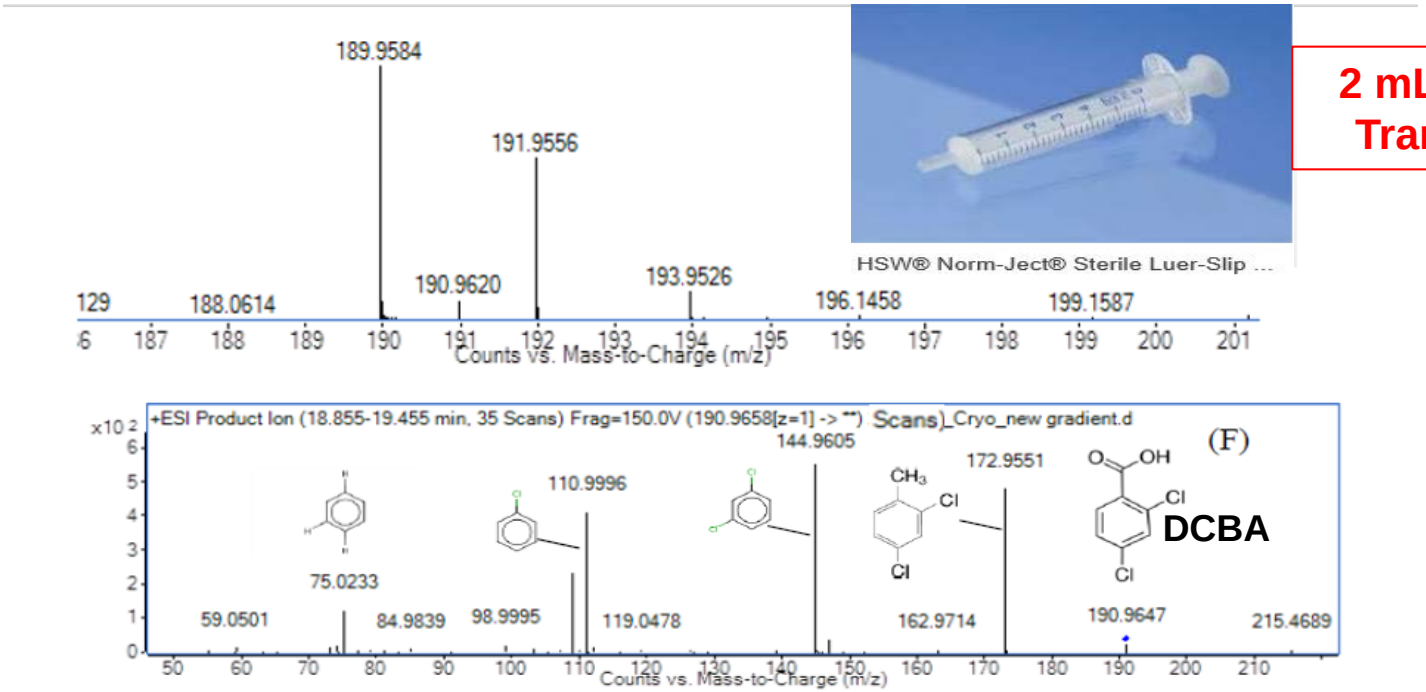
GMP Method Transfer Case Study 1

Mass spec analysis





2 mL NORM-JECT Transfer syringe

- The observed mass is 189.9584 Da.
- The observed isotopic peak distribution pattern matches well against the theoretical mass spectrum of C7H4O2Cl2.
- The MS/MS spectrum can also be assigned to each fragment of C7H4O2Cl2, which is possibly match with dichlorobenzoic acid.
- It has been reported that 2,4-dichlorobenzoic acid can be released from silicone coated tubes. Therefore, it is likely that 2,4-dichlorobenzoic acid is the impurity.

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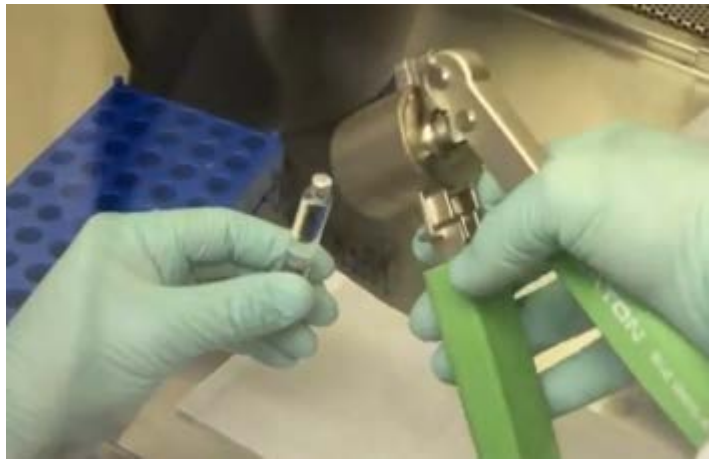
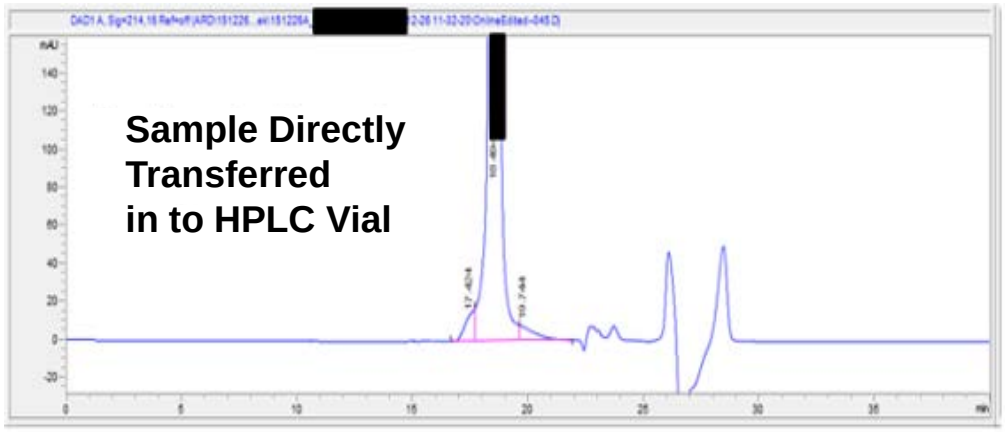
GMP Method Transfer Case Study 1



Direct Transfer




HSW® Norm-Ject® Sterile Luer-Slip ...

Waters Vials are certified clean using a 2-, 3-, or 4-step certification process:

	LC/GC Certified	LCMS Certified	TruView LCMS Certified
Dimensional Test	✓	✓	✓
Chemistry Test	✓	✓	✓
MS Scan		✓	✓
Low Adsorption Test			✓

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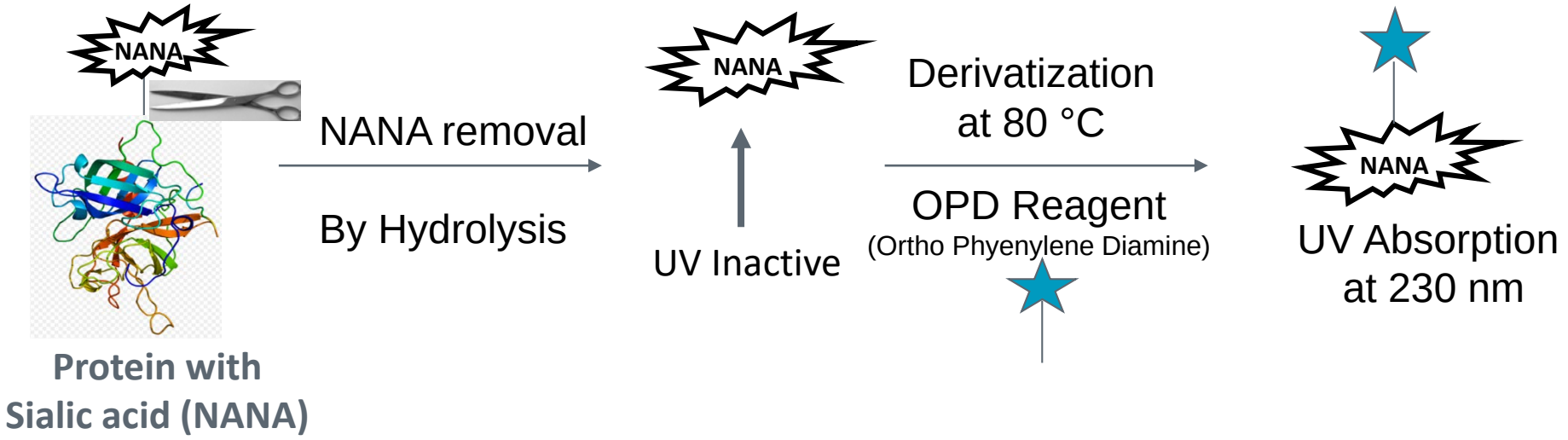
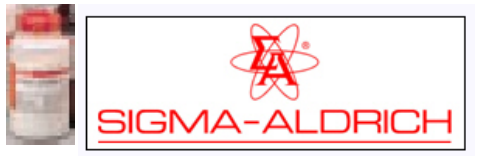
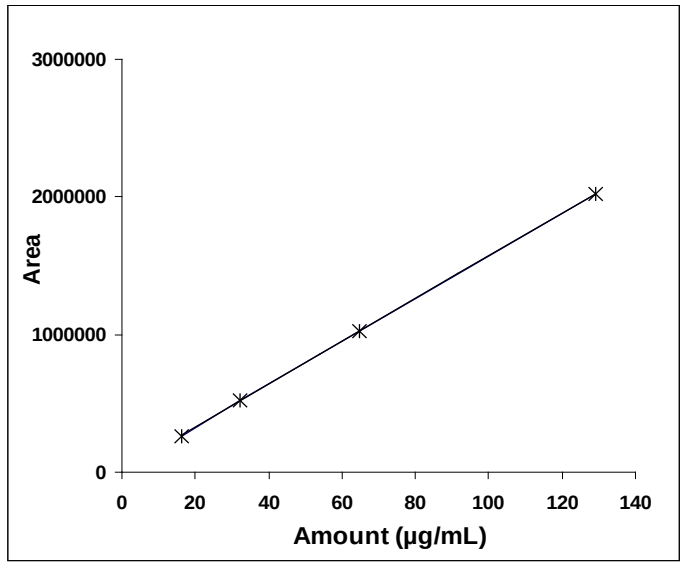
GMP Method Transfer Case Study 2



Protein with Sialic acid (NANA)



OPD-Commercially available and In-house qualified



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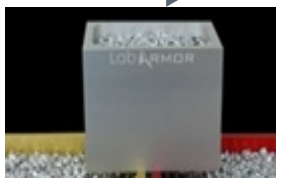
GMP Method Transfer Case Study 2

“DO NOT” expose to air



Time (mins)	Temperature of the solution °C **Target = 80±2 °C**	
	Aluminum Block/Beads (SEALED)	Aluminum Block/Lab Hormor Beads (OPEN)
0	22.8	22.3
1	48.5	50.9
2	63.3	60.0
3	71.5	64.1
4	75.4	67.3
5	77.6	70.1
6	79.0	71.7
7	79.3	72.6
8	79.6	73.8
9	79.9	74.2
10	80.0	75.4
11	80.1	75.3
12	80.1	75.6
13	80.0	76.6
14	80.2	76.8
15	80.1	76.7

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1. Lab Harmor beads or block should be sealed to avoid heat loss
2. The heat transfer significantly affects the reaction kinetics and derivatization

Thank you

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