BACHEM

ANALYTICAL TOOLBOX REQUIREMENTS

to enable the synthesis and release of large synthetic peptides

Date | April 9, 2024
Location | USP Workshop
Name | Tim Hellenbrand, PhD, Analytical Development

A LEADING SPECIALIST FOR DRUG SUBSTANCES

- Contract development and manufacturing organization (CDMO)
- Broad capabilities in Peptides and Oligonucleotides (TIDES) as active pharmaceutical ingredients (API)
- Long-term partnerships with pharmaceutical and biotech companies
- Focused on chemical synthesis, committed to innovation
- Annual sales of CHF 577.3 million in 2023 and over 2,000 colleagues globally
- Reliable supply of APIs for WHO essential medicines benefitting patients worldwide

High quality GMP manufacturing

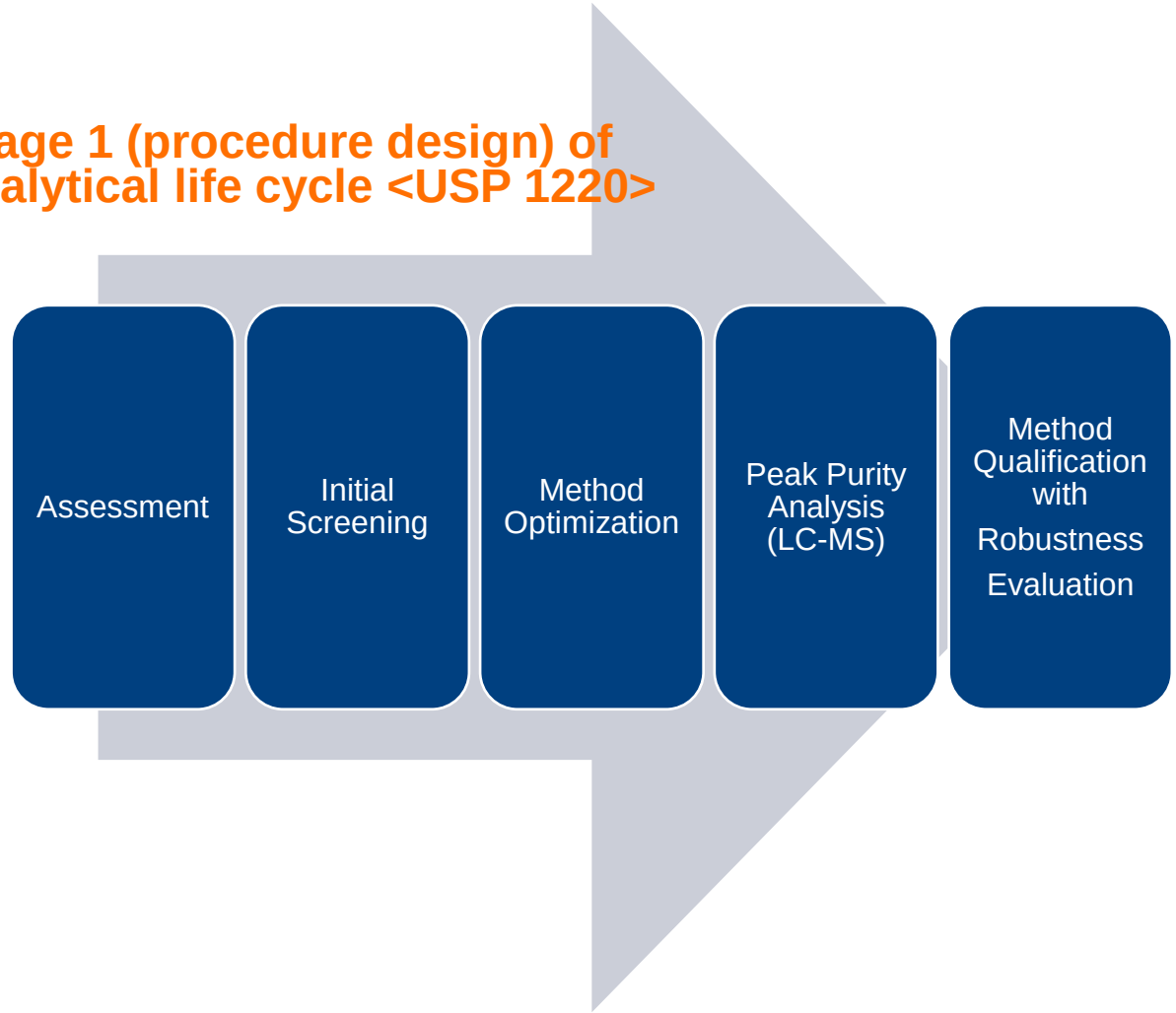
BACHEM

COMPLEX THERAPEUTIC PEPTIDES REQUIRE HIGHLY SELECTIVE PROCEDURES

- **Critical quality attributes (CQAs):**
  - Purity
  - Related impurities
  - Assay

- **Output:**
  - Identification of suitable procedure conditions
  - Understanding effect of procedure parameters
  - Initial analytical control strategy

Stage 1 (procedure design) of analytical life cycle <USP 1220>



BACHEM

ASSESSMENT FOR SYSTEMATIC USE OF PRIOR KNOWLEDGE

- **In-silico predictions of physico-chemical properties based on peptide sequence**

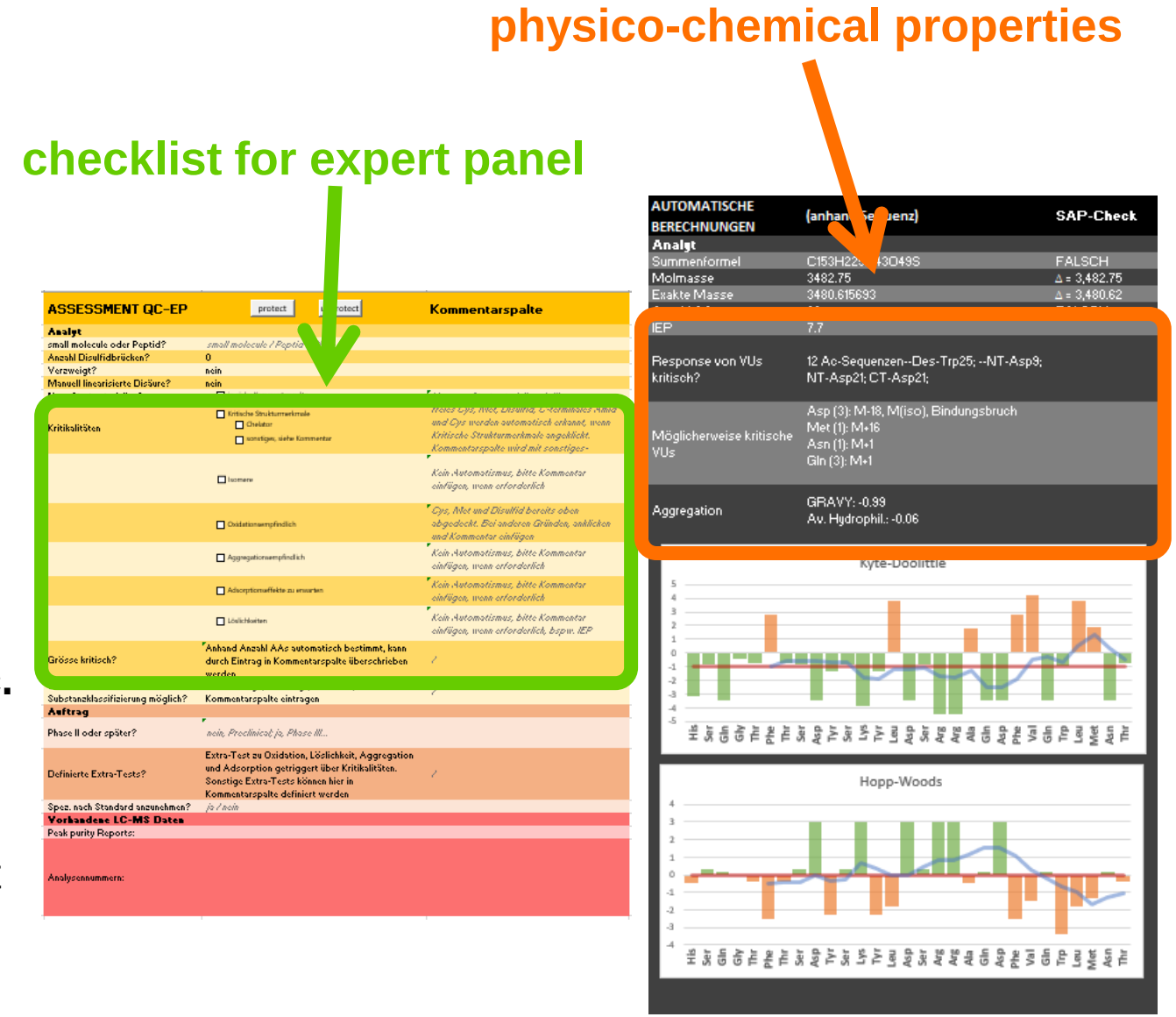
- **Expert panel evaluation based on molecular structure, synthesis route etc.**

➢ Predict impurities

➢ Oxidation/aggregation potential

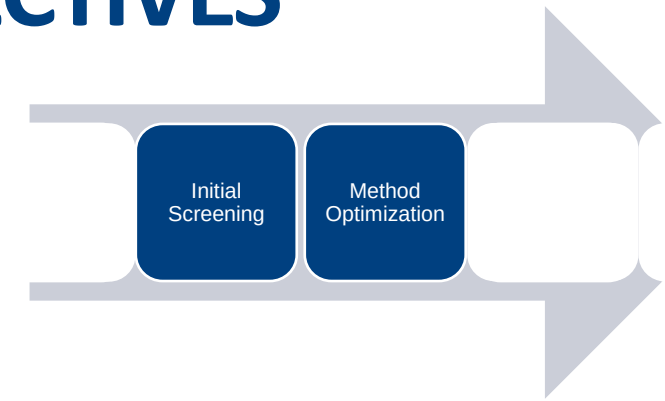
➢ Additional tests for procedure development

➢ Structural similarities with other APIs



BACHEM

DE NOVO METHOD DEVELOPMENT HAS MULTIPLE KEY OBJECTIVES



01 **Selectivity/Resolution**
limiting co-eluting impurities

02 **Sensitivity**
S/N ≥ 10

03 **Stability indicating**
detects related (degradation) impurities

04 **High-Resolution MS-compatibility**
choice and conc. additives

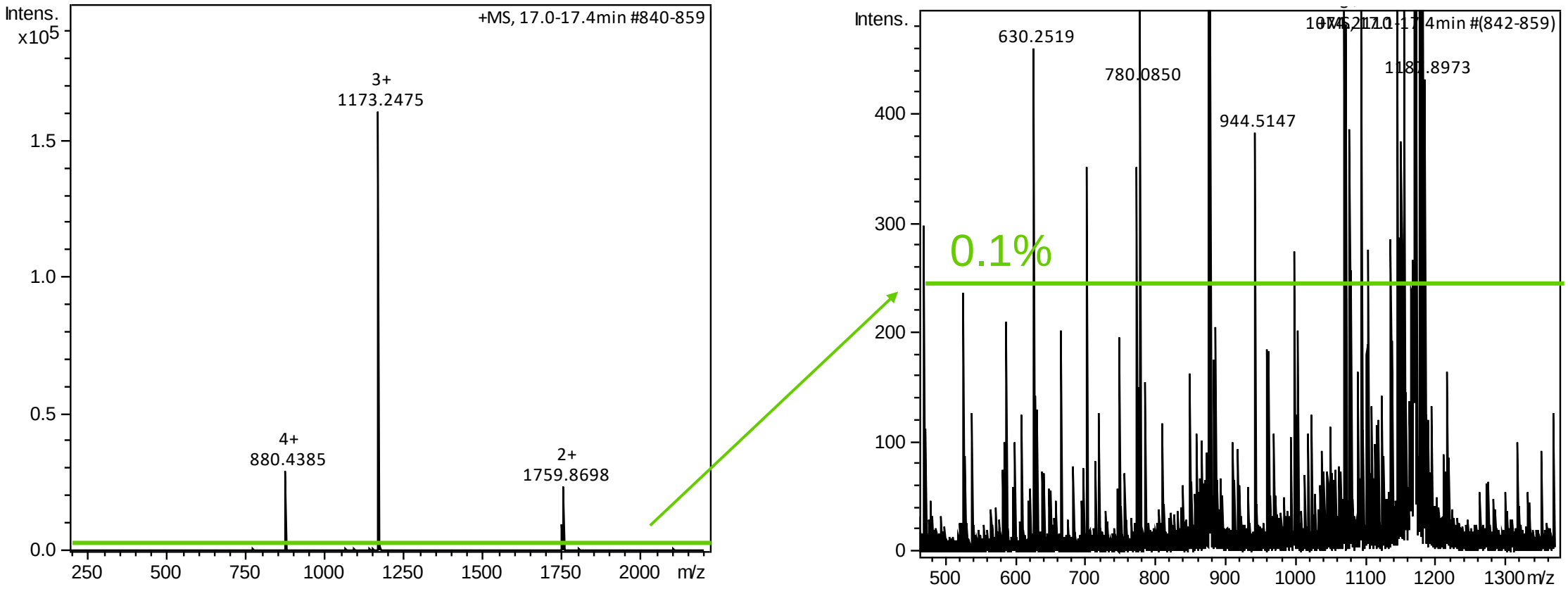
05 **Robustness & Applicability**
Reproducibility over time

06 **State of the Art**
Transferable to other labs

BACHEM

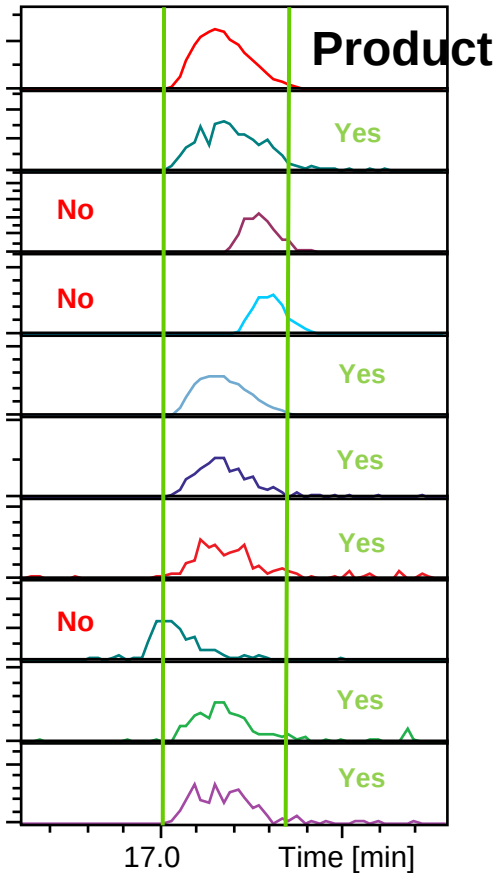
PEAK PURITY (LC-MS) FOR CO-ELUTING IMPURITIES

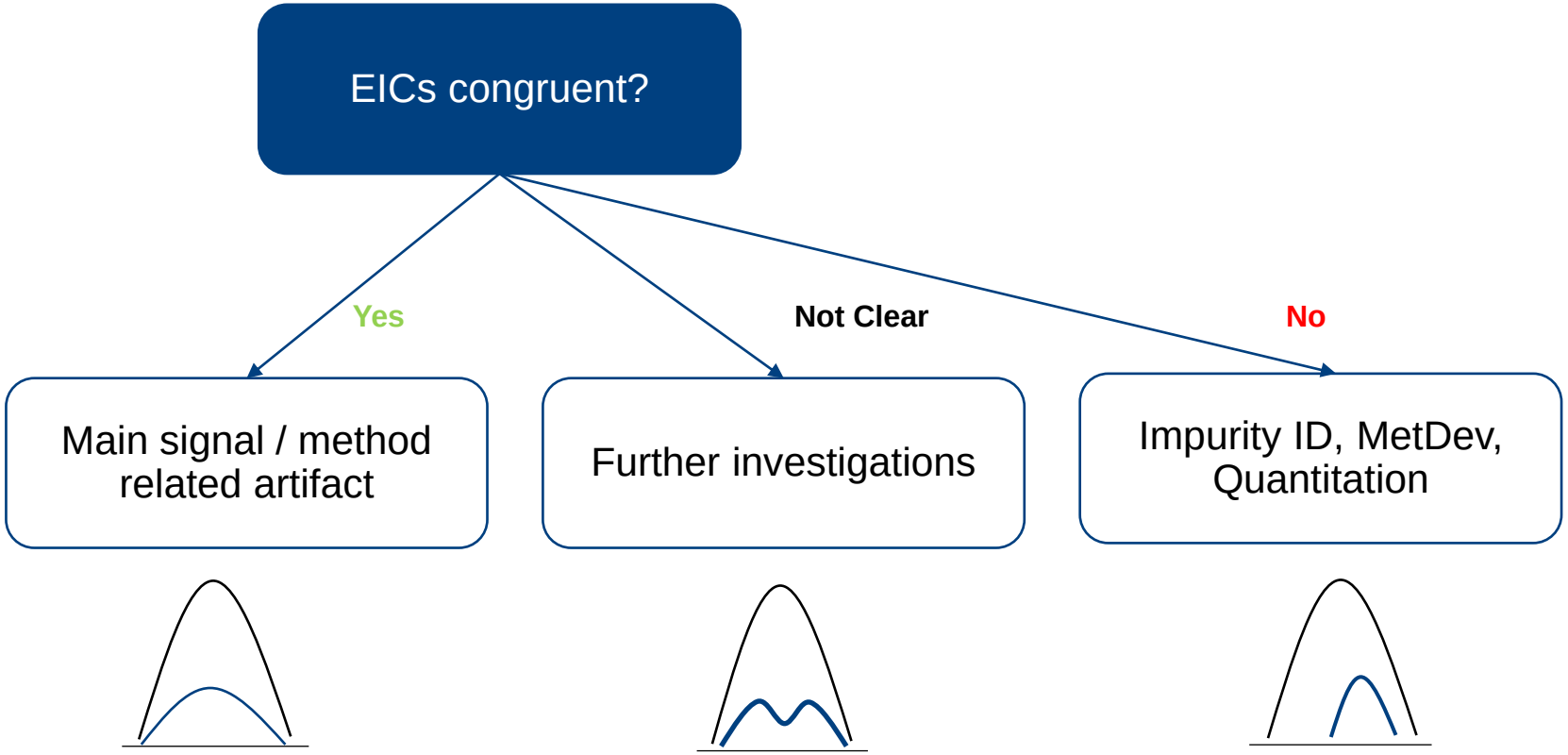
Average Mass Spectrum of Main Peak → Assignment of m/z values ≥ specified rel. intensity threshold → Automatic read-out of corresponding EICs



BACHEM

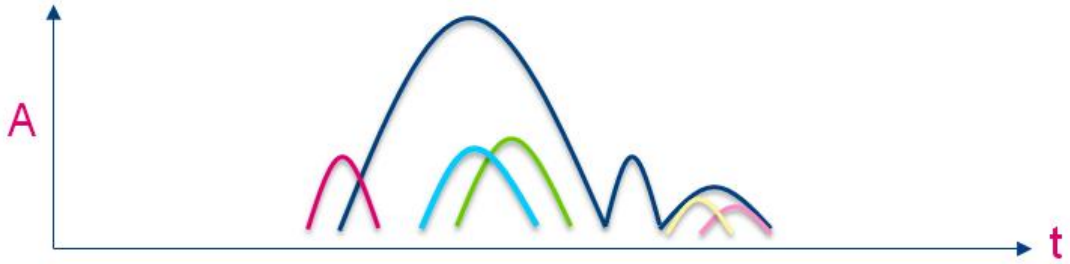
PEAK PURITY (LC-MS) FOR CO-ELUTING IMPURITIES





BACHEM

INITIAL REQUIREMENT
PURITY AND RELATED IMPURITIES



Co-elution of impurities with each other

summed UV peaks may exceed specified limits

⇨ unfavorable

Co-elution of impurities with main peak

Impurity may exceed specified limit without detection!

⇨ unfavorable

Identification of impurities

Identity might explain origin and stability behaviour

⇨ important

EP-Monograph 2034 „Substances for Pharmaceutical Use“

Table 2034.-2. – *Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis*

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

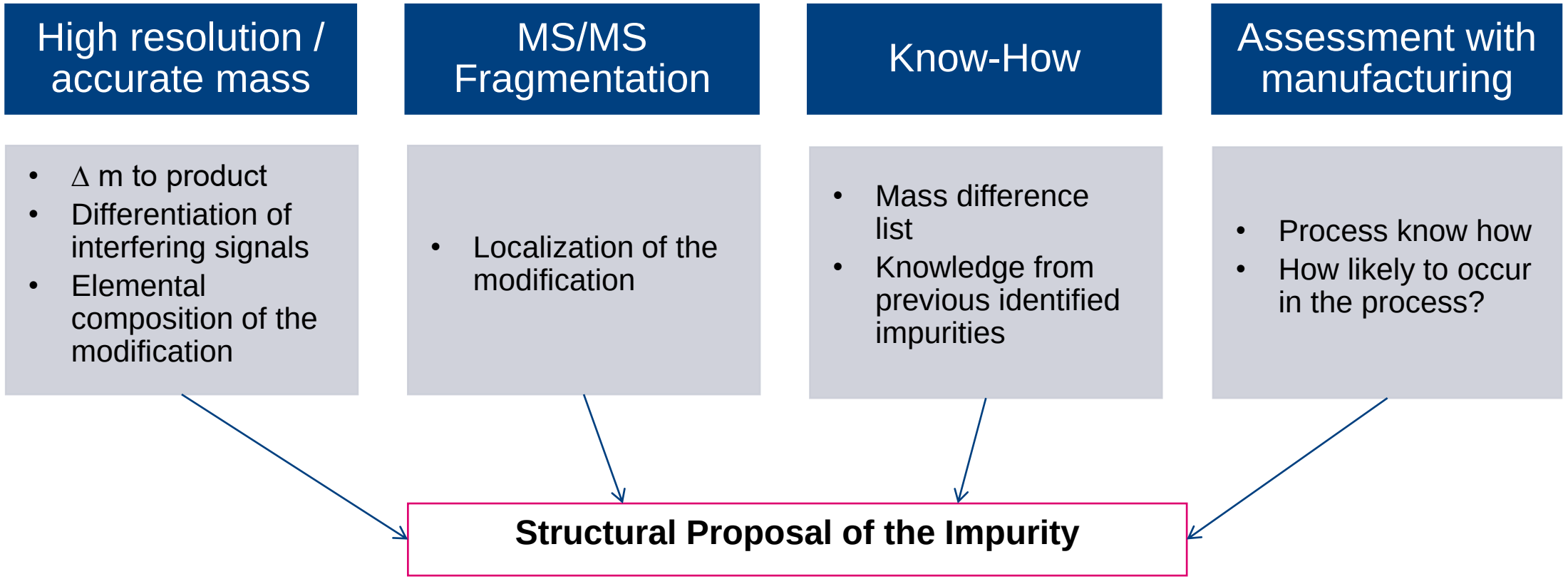
Purity: >95% (target)

Related Impurities:

- Specification for all impurities that may occur above 0.5%
- General limit for non-specified impurities < 0.5%
- Synthesis of impurity references

BACHEM

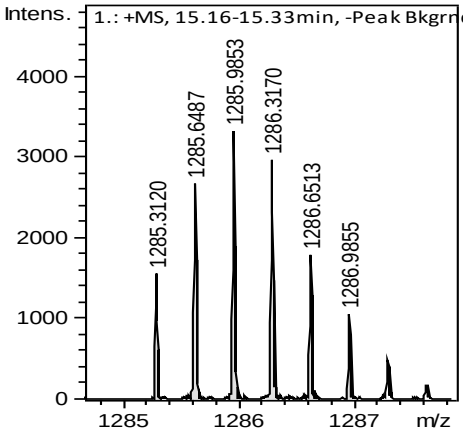
IDENTIFICATION BY LC-MS/MS – PROCEDURAL APPROACH



BACHEM

IDENTIFICATION BY LC-MS/MS
EXAMPLE OF UNKNOWN IMPURITY M+104

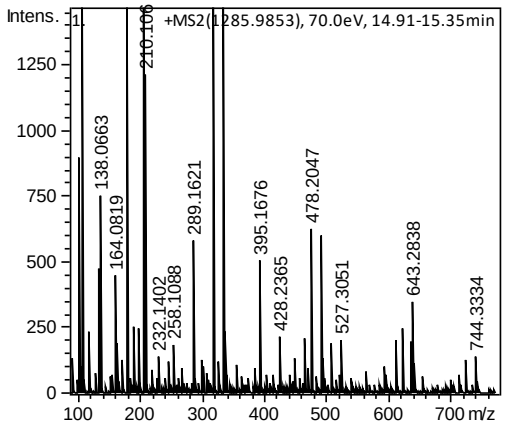
High resolution / mass accuracy



M+103.975 u

M+$C_3H_3S_2$

MS/MS Fragmentation



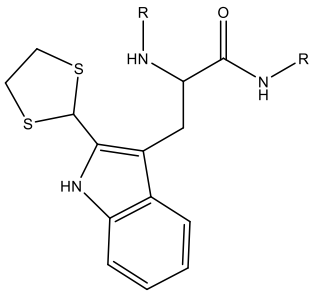
H-(1-24)-⌈Trp^{25}⌉-(26-31)-OH

Know-How

- Tryptophane sidechain on Indole Pos. 2’ →reactive!
- Similar species with additional TFA-Adduct previously found

Assessment with manufacturing

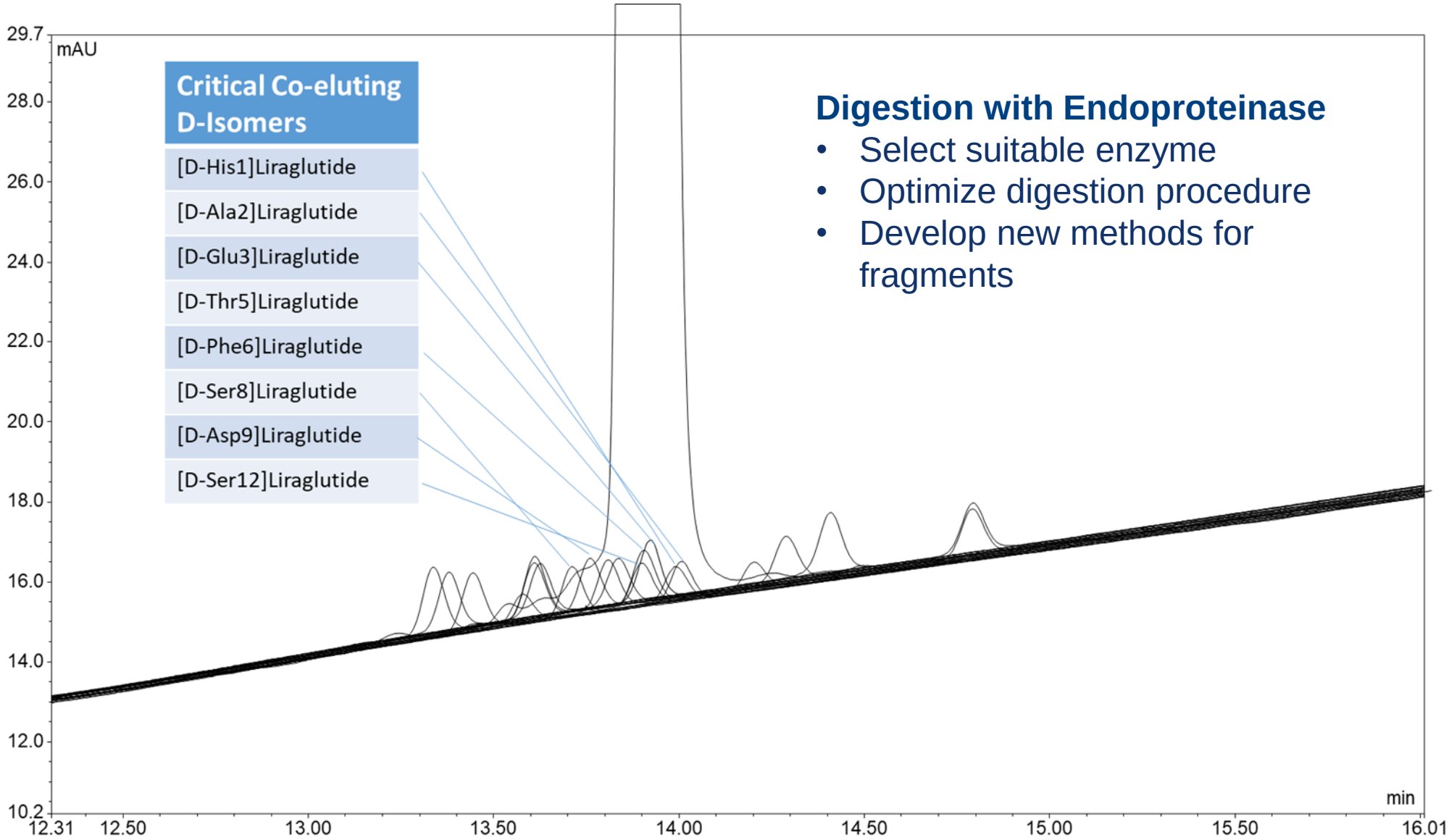
- EDT used in process
- Literature screening for modification of tryptophan residues



[2-(1,3-Dithiolan-2-yl)-Trp^{25}]-Liraglutide

BACHEM

STEREOISOMERS POSE A CHALLENGE FOR LIRAGLUTIDE



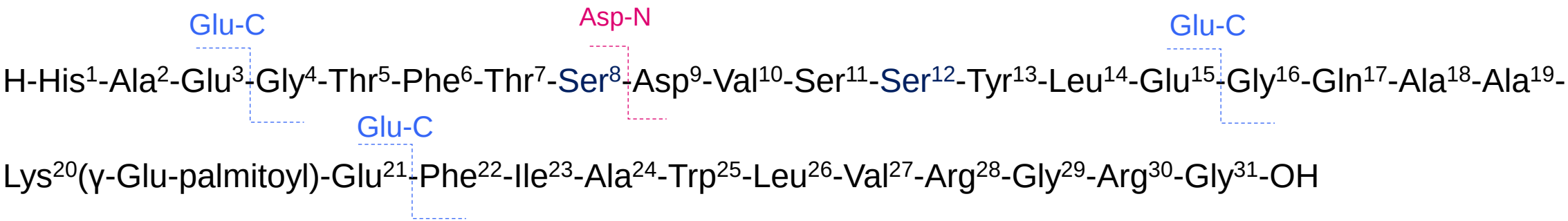
Digestion with Endoproteinase

- Select suitable enzyme
- Optimize digestion procedure
- Develop new methods for fragments

BACHEM

ENZYMATIC METHODS FOR D-ISOMERS

Liraglutide



Asp-N (Fragment 1-8)

[D-His1]Liraglutide
[D-Ala2] Liraglutide
[D-Glu3] Liraglutide

[D-Thr5] Liraglutide

[D-Phe6] Liraglutide

Glu-C (Fragment 4-15)

[D-Ser8] Liraglutide

[D-Asp9] Liraglutide

[D-Ser12] Liraglutide

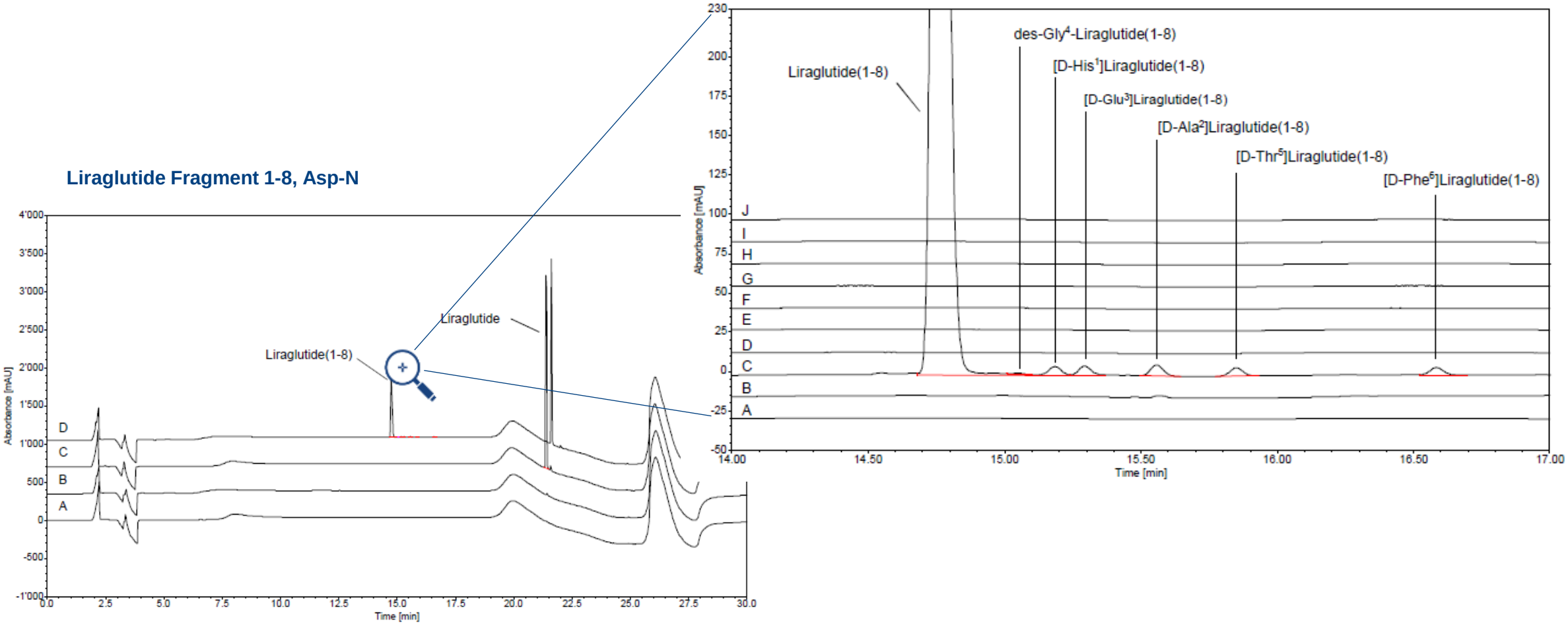
3rd impurity method (w/o enzyme)

[D-Glu15] Liraglutide
[D-Lys20] Liraglutide
[D-Glu21] Liraglutide
[D-Phe22] Liraglutide

After optimization of digest and development of HPLC methods, spiking experiments were conducted to identify the D-isomers fragments

BACHEM

HPLC METHOD FOR D-ISOMERS OF ASP-N DIGEST



Overlay of full scale chromatograms of a blank (undigested) (A), a blank (digested) (B), Liraglutide spiked with 0.5% of all six specified impurities (undigested) (C) and Liraglutide spiked with 0.5% of all six specified impurities (digested) (D)

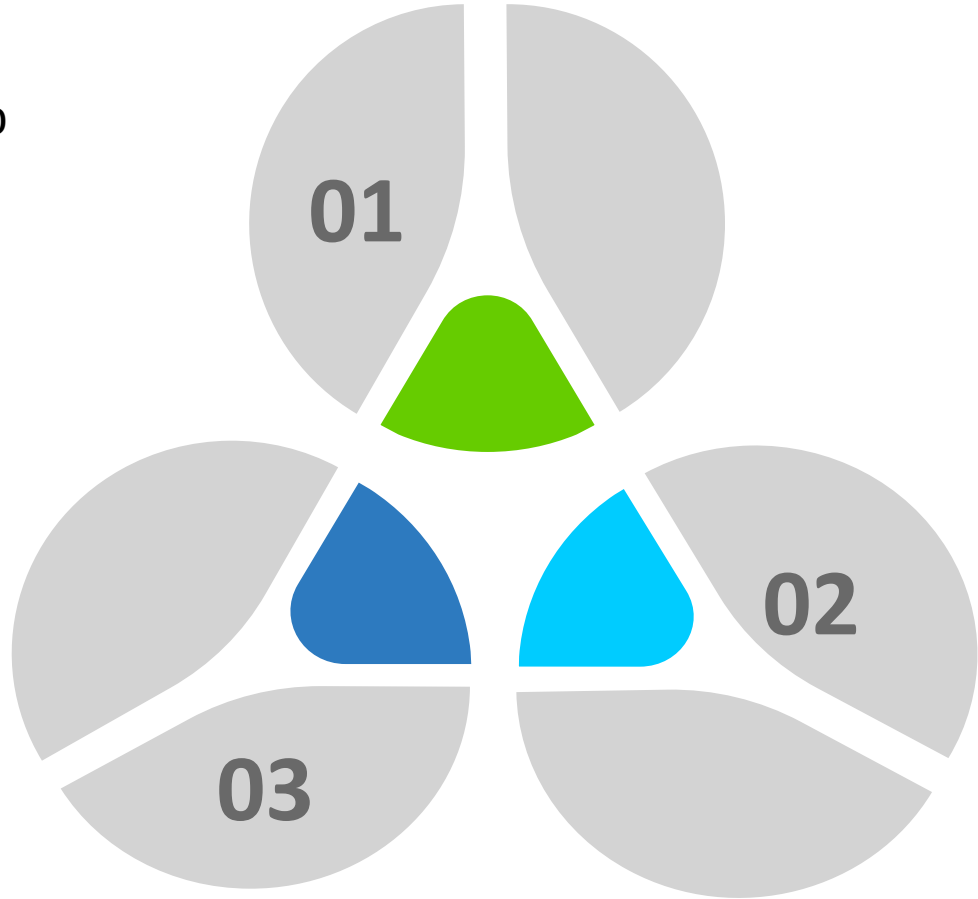
BACHEM

BATCH RESULTS WITH ENZYMATIC METHODS

	Batch	[D-His1] [%]	[D-Ala2] [%]	[D-Glu3] [%]	[D-Thr5] [%]	[D-Phe6] [%]	[D-Ser8] [%]	[D-Ser12] [%]	[D-Glu15] [%]	[D-Glu21] [%]	[D-Lys20] [%]	[D-Phe22] [%]
PPQ	Batch 9	0.13	<	<	<	<	0.27	0.06	<	<	<	0.07
	Batch 8	0.13	<	<	<	<	0.28	0.07	<	<	<	0.06
	Batch 7	0.13	<	<	<	<	0.27	0.07	<	<	<	<
Pr.Dev	Batch 6	0.10	<	<	<	<	0.26	0.07	<	<	<	0.14
	Batch 5	0.11	<	<	<	<	0.27	0.06	<	<	<	0.14
	Batch 4	0.11	<	<	<	<	0.27	0.07	<	<	<	0.13
	Batch 3	0.12	<	0.05	<	<	0.30	0.08	<	0.05	<	0.16
	Batch 2	0.15	0.06	0.05	<	<	0.29	0.07	<	0.05	<	0.06
	Batch 1	0.19	0.07	0.07	<	<	0.30	0.08	0.06	<	<	0.07
RLD	GS63P81	0.70	<	<	<	0.13	1.23	0.47	<	0.05	0.05	<
	JS69S28	0.47	<	0.41	<	0.09	1.20	0.45	<	<	<	<
	JS67Y95 (EU)	0.40	<	<	<	0.14	1.29	0.47	<	<	<	<
	JS68C66 (USA)	0.34	<	<	<	0.16	1.33	0.50	<	0.05	<	<
	JS68L68 (USA)	0.29	<	<	<	0.15	1.25	0.45	<	0.05	<	<
	JS67T64 (USA)	0.37	<	<	<	0.15	1.36	0.50	<	0.06	<	<

ANALYTICAL TOOLBOX FOR PHASE III

Output:
Set of orthogonal methods to determine purity, related impurities & assay



HPLC Purity Method

- Determine purity of material
- Identify related impurities by MS
- Quantify related impurities by LC UV
- Determine assay by HPLC

LC MS Quantitation

- Co-eluting impurities unequivocally identified and quantified
- Impurities under main peak can be quantified
- Necessary when selectivity can not be achieved chromatographically

Enzymatic Digest

- Reduce complexicity of large peptides
- Resolve isomers that are challenging to detect by LC-MS/MS
- Quantify impurities by LC-UV

BACHEM

REQUIREMENTS FOR PHASE III

01

Purity
Purity ≥ 99.0% target

02

Related Impurities
Report impurities ≥ 0.05 %
Identify impurities ≥ 0.10 %

03

Specificity
specified impurities synthesized

04

Process Understanding
Origin and fate of impurities better understood

05

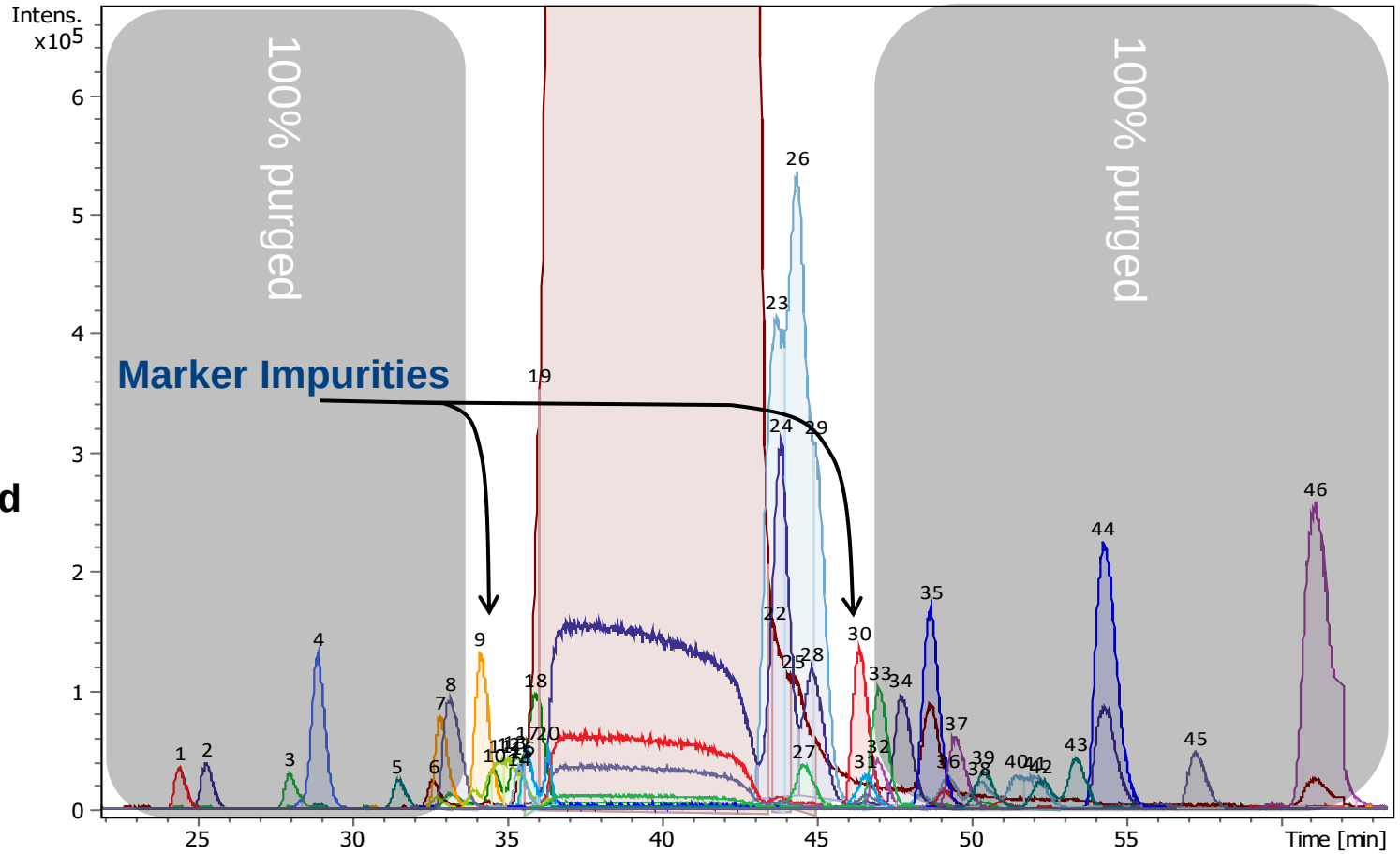
Comprehensive
methods enable detection of any Impurity or degradation product

06

Robustness
methods developed and optimized to ensure reproducible results

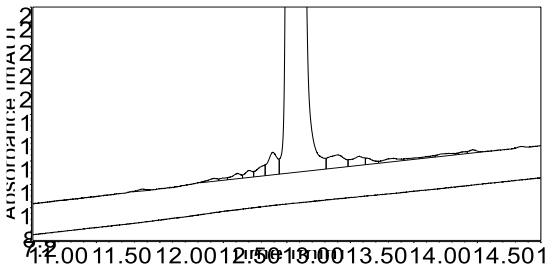
PREP LC-MS FATE & PURGE
HIGH EFFICIENCY

- **Impurities identified by MS and spiking experiments in case of isomers**
- **Define critical impurities for fate & purge**
- **The data set allows definition of regions where purge factors of 100% are achieved**
- **No synthesis and fate and purge study will be necessary for unambiguously assignable substances**



BACHEM

LIRAGLUTIDE PURITY METHOD



Liraglutide

0.10% (RRT0.981)
[Trp(O)^{25}]Liraglutide
Iso-Liraglutide

0.28% (RRT0.990)
[Kyn^{25}]-Liraglutide
D-Ser^{8}-Liraglutide

0.27% (RRT1.026)
[1,3-Dithiolan-2-yl-Trp^{25}]
Liraglutide
des-Ser^{8}-Liraglutide
des-Gly^{29}-Liraglutide

0.15% (RRT1.039)
[2-Trifluoromethyl- 1,3-dithiolan-2-yl-Trp^{25}]Liraglutide
[t-Butyl-Trp^{25}]Liraglutide
des-Gly^{31}-Liraglutide

0.07% (RRT0.973)
[Trp(2O)^{25}]Liraglutide

0.13% (RRT1.045)
unknown

0.05% (RRT0.965)
endo-Gly-Liraglutide

0.06% (RRT1.065)
des-Tyr^{13}-Leu^{14}-
Liraglutide

0.07% (RRT0.950)
[Trp(O)^{25}]Liraglutide

des-Gly^{4}-Liraglutide
D-His^{1}-Liraglutide

Reporting threshold: 0.05 %

BACHEM

LIRAGLUTIDE BATCH COMPARISON

Batch	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6		Batch 7		Batch 8	
Assignment	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**	**% Area[(1)]**	**Δm**
RRT 0.950	0.07%	+16	<	-	0.06%	+16	<	-	<	-	<	-	<	-	<	-
RRT 0.96	<	-	<	-	0.05%	+57	0.05%	+57	<	-	<	-	<	-	<	-
RRT 0.973	0.07%	+32	0.07%	+32	0.06%	+32	0.06%	+32	<	-	0.06%	+32	<	-	<	-
RRT 0.981	0.07%	+16	0.07%	+16	0.09%	+16	0.09%	+16	0.09%	+16	0.08%	+16	0.05%	+16	0.05%	+16
Impurity 5								±0		±0		±0				
RRT 0.990	0.20%	+4	0.17%	+4	0.18%	+4	0.23%	+4	0.25%	+4	0.11%	+4	0.09%	+4	0.08%	+4
Impurity 1								±0		±0		±0		±0		±0
RRT 1.026	0.27%	+104	0.20%	+104 (1)	0.19%	+104 (6)	0.23%	-87 (1)	0.11%	-87 (2)	0.19%	-87 (4)	0.11%	-87	0.11%	-87 (4)
				-87 (1)		-87 (1)		-57 (1)		-57 (1)		-57 (1)				-57 (1)
Impurity 2						-57 (1)										
RRT 1.039	0.14%	+172	0.12%	+172 (2)	0.10%	+172	0.12%	+172 (5)	0.15%	+172 (2)	0.08%	+172 (1)	0.05%	+172 (1)	0.07%	+172 (1)
Impurity 3				+56 (1)				-57 (4)		-57 (1)		-57 (1)		-57 (1)		-57 (1)
										+56 (1)				+56 (1)		+56 (1)
RRT 1.045	0.13%	+115	0.08%	+115	0.09%	+115	0.05%	n.d.	0.13%	n.d.	<	-	<	-	<	-
Impurity 4																
RRT1 .060/1 065	0.06%	-276	<	-	<	-	0.06%	-276	0.06%	-276	<	-	<	-	<	-
RRT1.110	<	-	<	-	<	-	<	-	<	-	<	-	0.07%	+14	0.05%	+14
RRT 1.234	0.06%	+3632	<	-	<	-	<	-	<	-	<	-	<	-	<	-
Purity [(1)]	**98.9%**		**99.3%**		**99.2%**		**99.1%**		**99.1%**		**99.4%**		**99.8%**		**99.7%**	

(1) Values taken from release analysis

LIRAGLUTIDE IMPURITY SPECIFICATIONS

RRT	Δm [u]	Proposed Structure	max. %area (UV) found	Present in RLD	Impurity specified
0.981	+16	[Trp(O)^{25}]Liraglutide	0.10%	Yes	**Specified impurity 5**
0.990	+4	[Kyn^{25}]Liraglutide	0.25%	No	**Specified impurity 1**
	±0	iso-Liraglutide, D-Ser8		Yes	
1.021 / 1.026	+104	2-(1,3-Dithiolan-2-yl-Trp^{25}]Liraglutide	0.27%	No	**Specified impurity 2**
	-87	des-Ser^{8}-Liraglutide		No	
	-57	des-Gly^{29}-Liraglutide		No	
1.039	+172	[2-Trifluoromethyl-1,3-dithiolan-2-yl-Trp^{25}]Liraglutide	0.15%	No	**Specified impurity 3**
	+56	[t-Butyl-Trp^{25}]Liraglutide		No	
	-57	des-Gly^{31}-Liraglutide		Yes	
1.045	+115	unknown	0.13%	No	**Impurity not present with final process**

RELEASE METHOD AGILITY AND EXPERTISE IS CRUCIAL FOR THE SUCCESS OF COMPLEX PEPTIDE THERAPEUTICS

- Complex Peptide NCEs require **product-specific method development**
- Quality control strategy **must fulfill the needs of various stakeholders**
- **Complex impurity profiles** need to be addressed with tailored analytics
- Tight **ANDA-driven** requirements can be met for large synthetic peptides

QUALITY MATTERS

Cutting-edge analytical capabilities ensure development success and patient safety

BACHEM

GREAT TEAM KEY TO SUCCESS

Analytical Development

Agron Selami, Michael Naeff, Patrik Plattner, Constanze Schmies, Jürgen Opitz





CMC / Projekt Management:

Michael Berger, Michael Wollmann


Review/Mentoring:

Roland Eberli

BACHEM

BACHEM

THANK YOU

bachem.com

Bachem AG
4416 Bubendorf
Switzerland

Tel +41 585 95 20 21

E-Mail
sales.ch@bachem.com

Bachem Americas, Inc.
Torrance, CA 90505
USA

Tel +1 888 422 24 36

E-Mail sales.us@bachem.com

Bachem Japan K.K.
Tokyo 103-0012
Japan

Tel +81 3 6661 0774

E-Mail sales.jp@bachem.com

UNDERSTANDING AGGREGATION BEHAVIOUR IMPORTANT FOR PROCESS DEVELOPMENT AND QC

QC Testing

HPLC SEC

- Determination of HMWP
- Quantitative, robust, sensitive (LOQ 0.1%)
- Routinely applicable for release
- MS suitable

Aggregation Screening

DLS

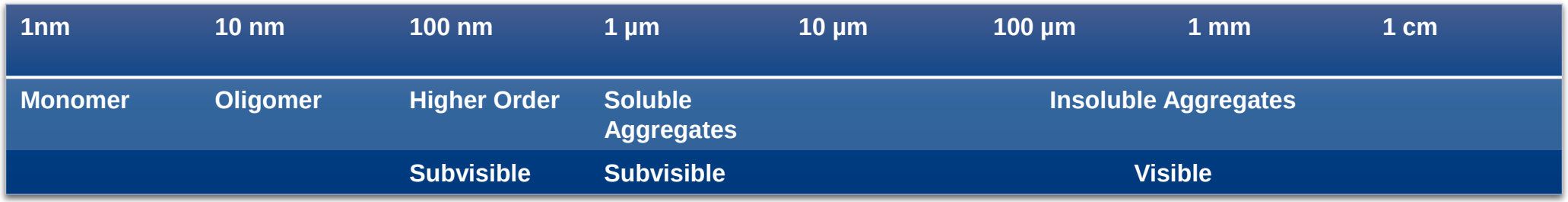
Measure kintetics to detect molecules with larger hydrodynamic radius

THT Assay

Linear fluorescence assay relating to the original amount of seeds

CD Spectroscopy

Detect secondary structures
Comparative measurement API formulation / RLD



BACHEM