Catalent BIOLOGICS

Evaluating the Stability of Monoclonal Antibodies: Forced Degradation in Method Development

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Broad Capacity, Extensive Technical Expertise & Exceptional Customer Experience

95%+

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- 9+ out of 10 customers would recommend to others
- Dedicated PM & technical lead

30+

Years of Experience

- 40% of analysts has MS or PhD
- 300+ programs supported
- Avg. 30 audits performed annually
- Zero 483-form issued during last inspection

200+

Scientists

- 800+ assays and techniques
- 20,000+ ft^3 of lab space
- 31,000+ ft^3 stability storage
- Low turnover ~10%

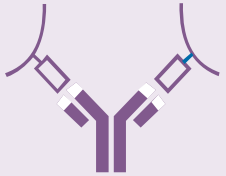
A Powerful Offering Stemming Across Modalities

From Traditional to Emerging Modalities & from Discovery to Commercial

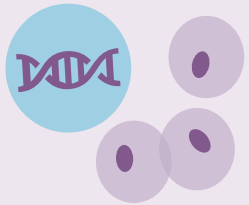
mAbs & Bispecific Abs

Pegylated Peptides

Bioconjugates & ADCs



Recombinant, Fusion Proteins



Gene & Cell Therapies

Nucleic Acids

Supports All Modalities

Discovery → Preclinical → Phase I → Phase II → Phase III → Commercial

- Developability & pre-formulation studies
- Comparability studies
- Extended molecule and product characterization
- Method development
- Phase-appropriate method qualification
- Phase-appropriate method validation
- Compendial method verification
- Stability studies
- Material and impurity characterization and identification
- GMP release testing

AGENDA

1. **Why Forced Degradation Matters**
2. Major Degradation Pathways
3. Forced Deg Study: Cetuximab
4. Execution: Forced Degradation Results
5. Your Molecule at Catalent
6. Q&A

The Problem with Stability in mAbs

What if your biologic fails stability testing late in development?

Reformulation

Shorter shelf life

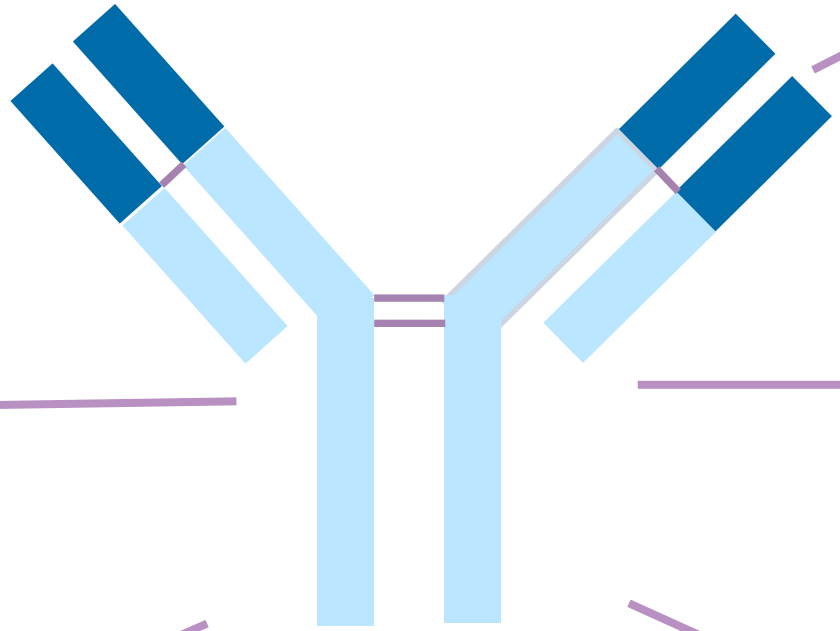
Additional testing

Regulatory delays

Increased costs

Risk of project termination

Understanding Forced Degradation (FD)



- Understanding Stability Profiles
- Formulation Development
- Method Validation and Development
- Quality by Design (QbD)
- Regulatory Compliance
- Shelf-Life Prediction
- Safety Considerations

Why Forced Degradation Matters

Accelerated forced degradation mimics long-term storage conditions, providing knowledge into potential degradation mechanisms during a product's shelf-life

Quality by Design	Helps define the design space to maintain critical quality attributes
Chemical Stability	Identifies pathways like deamidation, oxidation, aggregation, and fragmentation
Physical Stability	Evaluates the impact of temperature, pH, UV/light, freeze/thaw, and agitation on antibody structure

Why Forced Degradation Matters (cont.)

Regulatory Compliance	Meets FDA, EMA, and ICH Q1A guidelines to confirm stability-indicating methods
Analytical Specificity	Develops methods to detect impurities and degradation products.
Formulation Development	Identifies excipients that affect stability to optimize formulation
Safety Considerations	Assesses degradation products for potential safety risks at clinical levels

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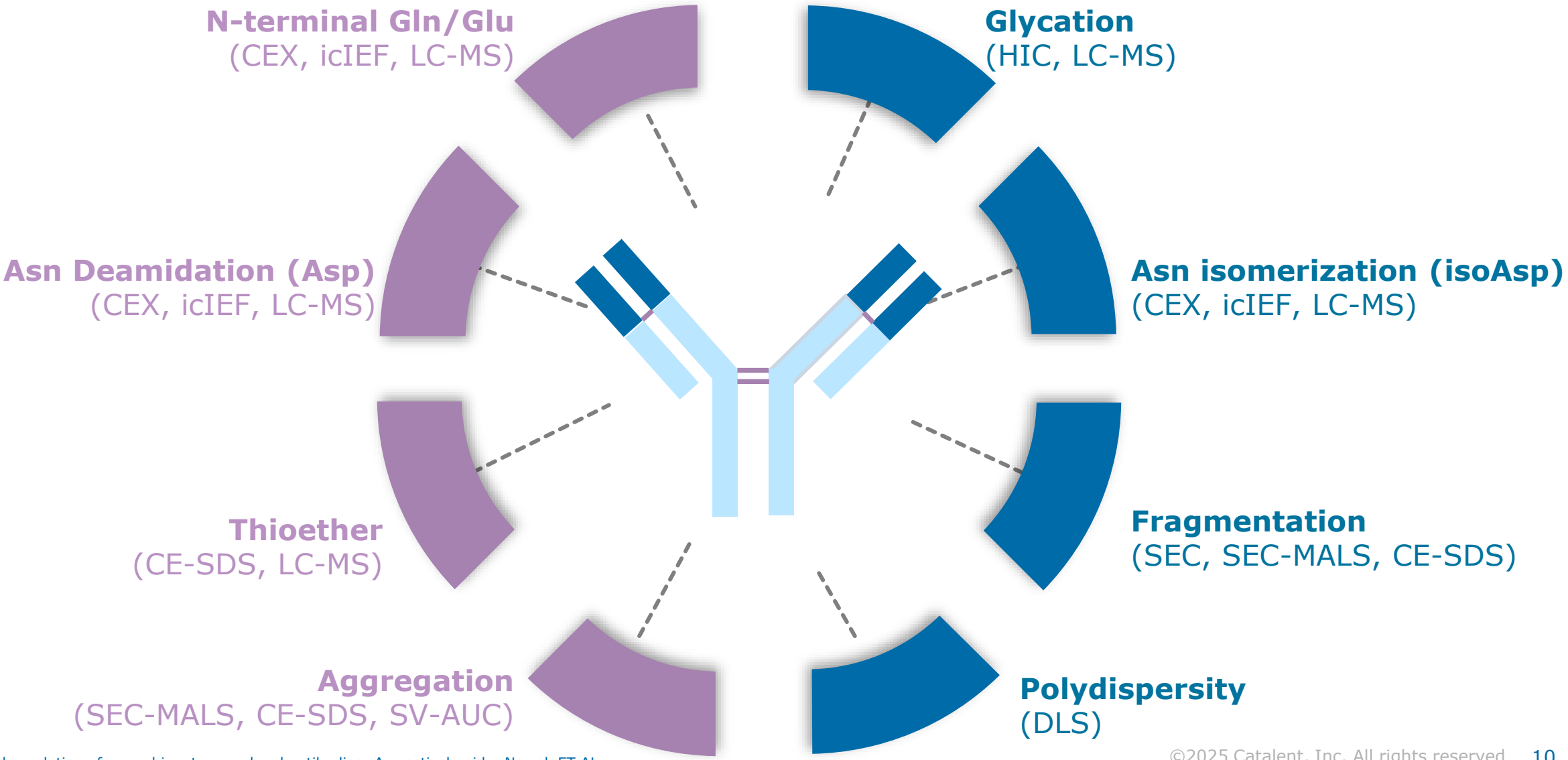
Typical Degradation Pathways of mAb's



Forced degradation of recombinant monoclonal antibodies: A practical guide, Nowak ET AL.

Analytical Instrumentation for Biologics Development

SV-AUC

- MW, sedimentation coefficient, % distribution
- Ensures aggregation & stability analysis

SEC-MALS

- MW, size & polydispersity
- Provides purity & consistency profiles

Maurice icIEF

- Real-time charge heterogeneity analysis
- Supports robust QA/QC processes

HPLC/ UPLC

- High-resolution purity & potency analysis
- Ensures biologic quality & performance

Image source: Images of analytical instruments are from respective vendors and are for illustrative purposes only. All trademarks and product names are the property of their respective owners.

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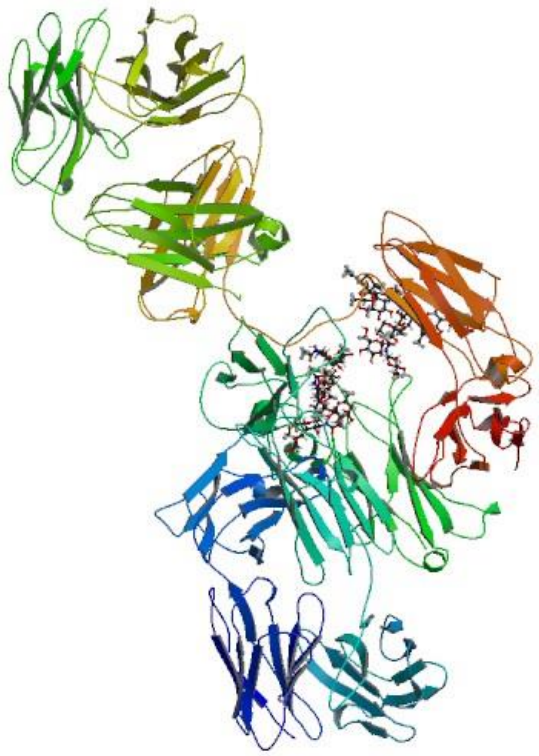
Forced Degradation Study: Cetuximab



Indicated for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer as well as head and neck cancer

Image source: DRUGBANKOnline

- **Cetuximab** was chosen for its significance as a therapeutic **IgG1 mAb**
- Formulated at **10 mg/mL** in 10 mM sodium phosphate, 140 mM NaCl; pH 7.2; pI = 8.48

- Forced degradation studies require **advanced bioanalytical capabilities to accurately detect, characterize & quantify molecular changes** under various stress conditions

Analytical Methods Employed for FD Study

SEC-MALS
(Molecular weight, aggregation, fragments, non-covalent association)

SV-AUC
(Sedimentation coefficient, Size distribution, molecular weight, self-association, irreversible aggregation)

DLS
(Monodispersity, polydispersity, size distribution)

icIEF
(PTM, charge variants, heterogeneity, pI)

CE-SDS
(Covalent aggregates, purity, cross-linking)

HIC
(Oxidation, deamidation, aggregation)

CEX
(Deamidation, oxidation, glycation)

Degradation Conditions Used for Study

- **Chemical Degradation**
  - pH (acid & base)
  - Oxidation
  - Photolysis
- **Physical Degradation**
  - Thermal
  - Freeze / thaw
  - Mechanical (stir / shake)

Test	Chemical Degradation				Physical Degradation			
	Hydrolysis		Oxidation (H_2O_2)	Photolysis (UV 15 hrs)	Thermal (50°C)	Freeze/Thaw (5 cycles)	Mechanical	
	Acid (pH 3)	Base (pH 9)					Stir (magnetic)	Shake (350 rpm)
HIC			X		X			
CEX	X	X			X			
DLS	X	X		X	X	X	X	
SEC-MALS	X	X		X	X	X	X	
SV-AUC	X	X		X	X	X	X	
icIEF	X	X	X	X	X			
CE-SDS	X	X			X			

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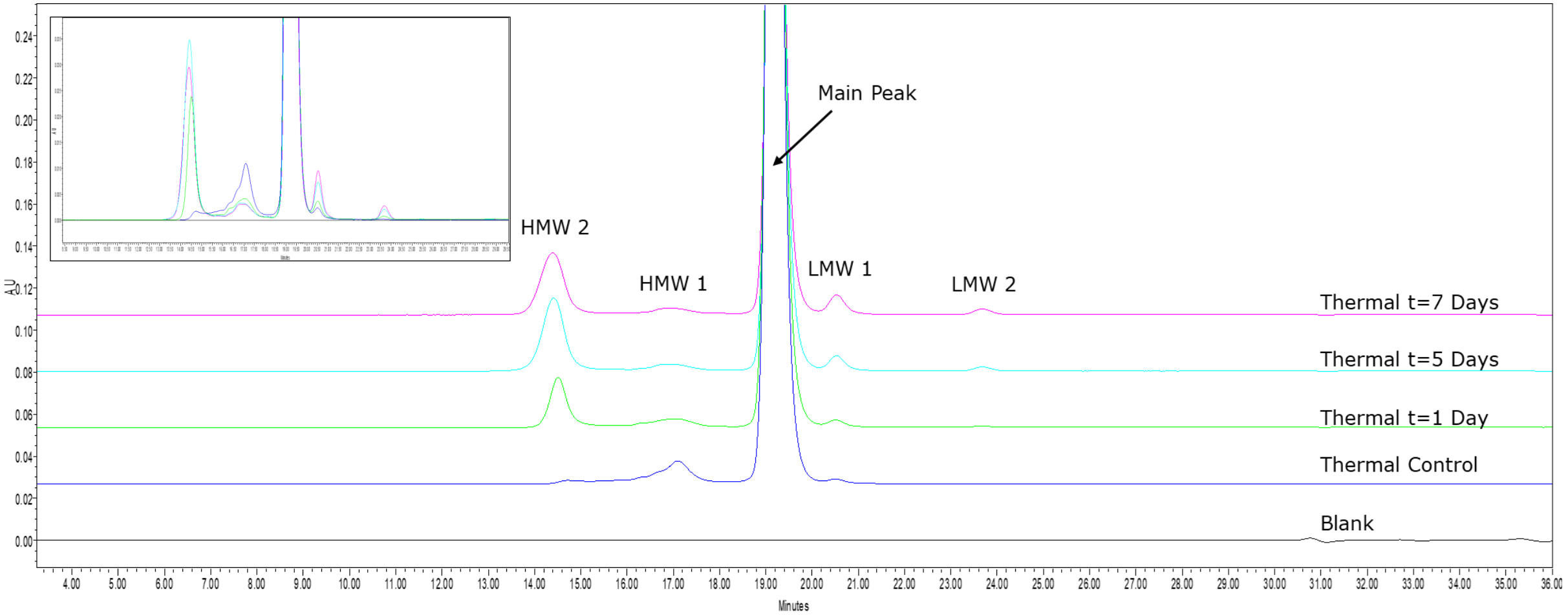
SEC-HPLC Chromatograms (A_{280}) of Cetuximab Subjected to **Thermal** Degradation at 50°C for Up to 7 Days

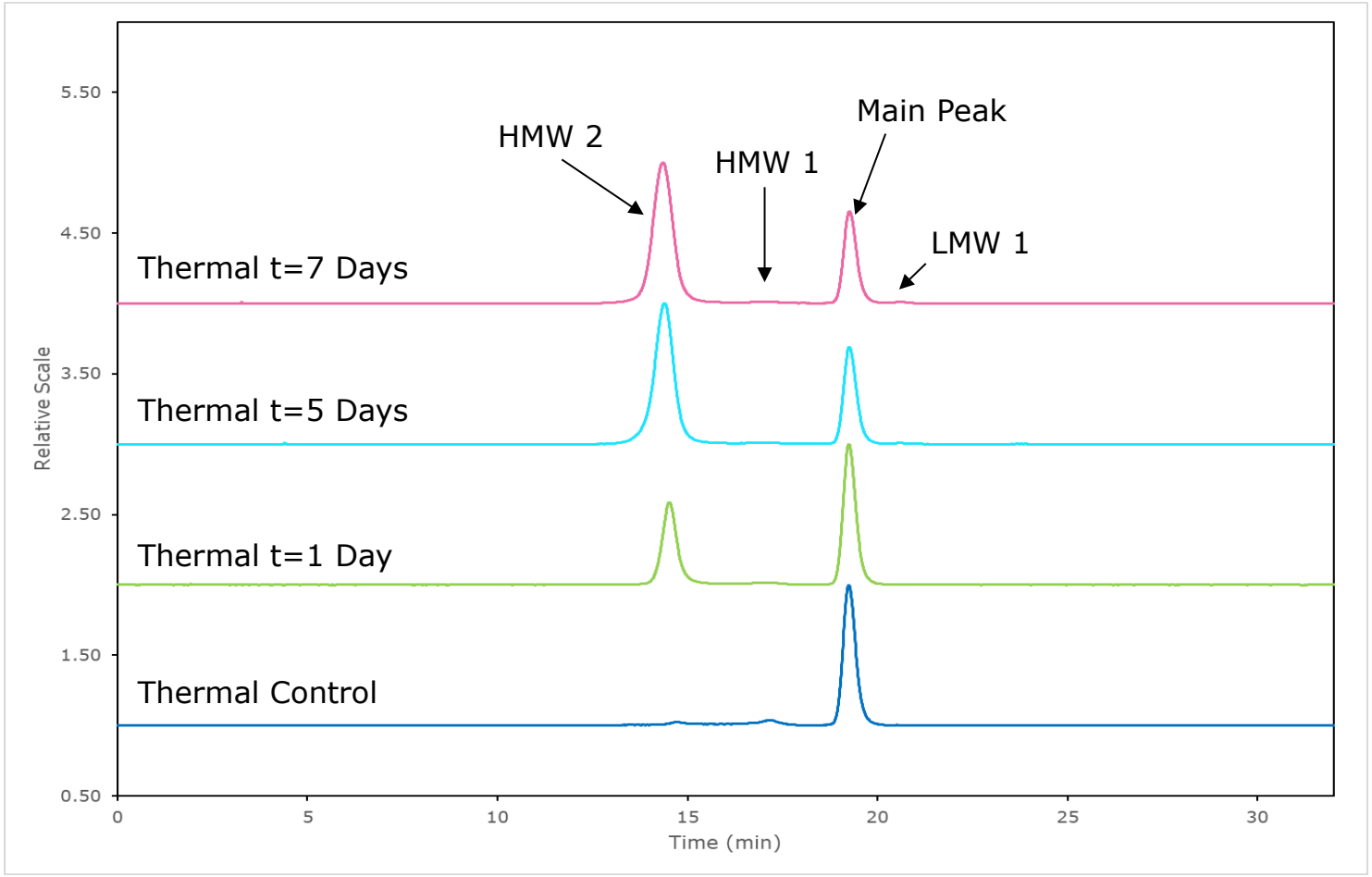


- Growth of HMW 2 species apparent even after 1 day at 50C
- Increase in fragment species (LMW 1, LMW 2) over the time course study

SEC-MALS Chromatograms & MW Data for Cetuximab Subjected to **Thermal** Degradation at 50°C Over 7 Days

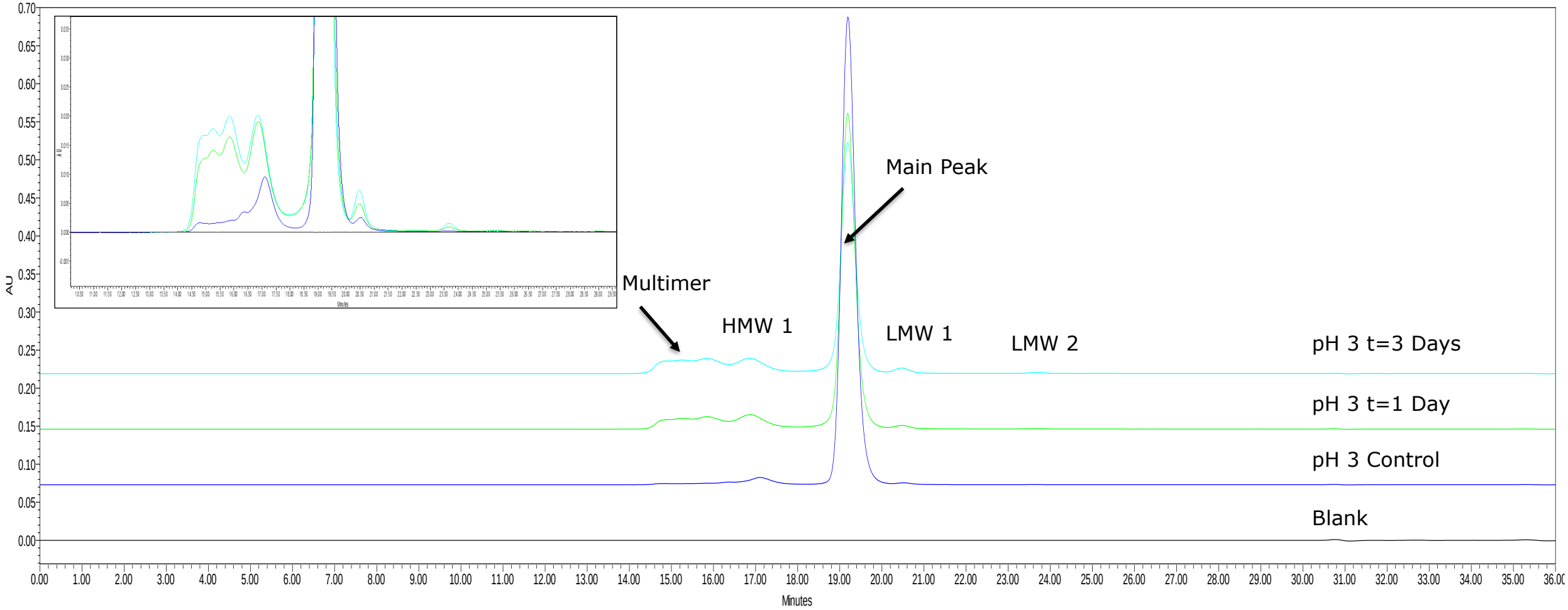
Sample ID/Lot	MW Monomer (avg), Da	MW HMW 1 (avg), Da	MW HMW 2 (avg), Da	MW LMW (avg), Da
Thermal Control	145800	276100	1095000	108200
Thermal T = 1 Day	145800	297100	1934000	93270
Thermal T = 5 Days	145800	332300	2451000	108000
Thermal T= 7 Days	145800	328100	2579000	106100

Sample ID/Lot	PD, Monomer (Mw/Mn)	PD, HMW 1 (Mw/Mn)	PD, HMW 2 (Mw/Mn)	PD, LMW (Mw/Mn)
Thermal Control	1.000	1.001	1.097	1.105
Thermal T = 1 Day	1.000	1.005	1.051	1.043
Thermal T = 5 Days	1.000	1.009	1.090	1.033
Thermal T= 7 Days	1.000	1.005	1.107	1.011



- Thermal exposure displays a steady increase in HMW 2 species over the study
- Apparent increase in MW as well as polydispersity over 7 days at 50C seen in the HMW 2 peak

SEC-HPLC Chromatograms Showing **Acidic** Degradation of Cetuximab at pH 3 Over 3 Days

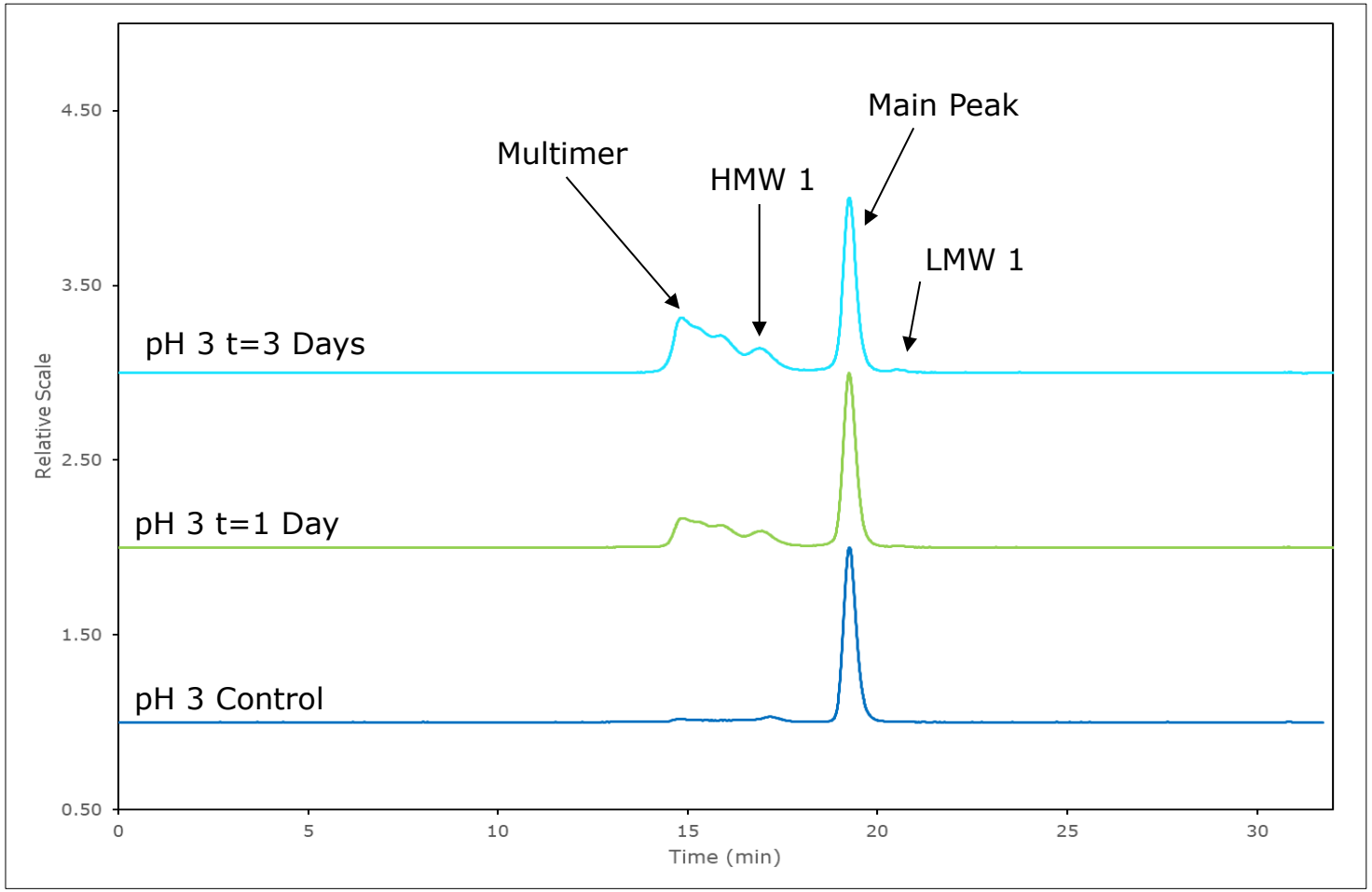


- Multimer species contains numerous variants due to acidic degradation
- Increase in fragment species (LMW 1, LMW 2) similar to thermal degradation

SEC-MALS Chromatograms & MW Data for Cetuximab Subjected to **Acidic** Degradation at pH 3 Over 3 Days

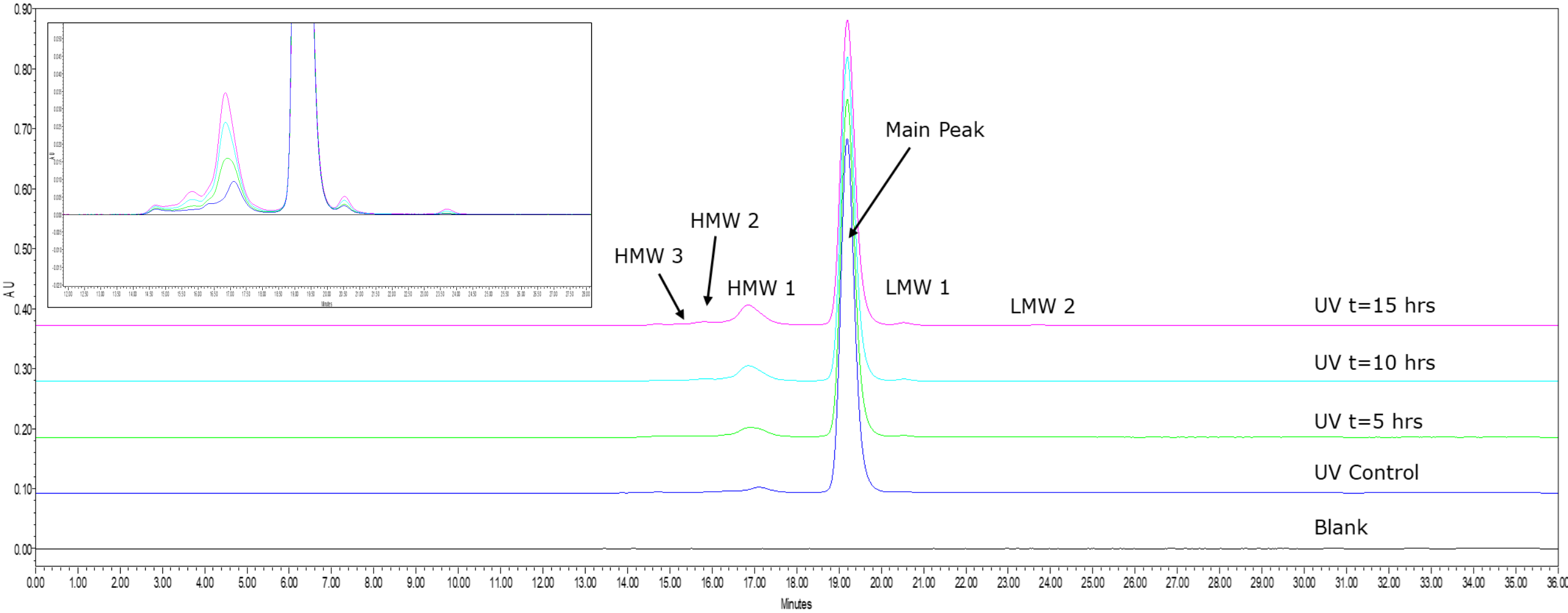
Sample ID/Lot	MW Monomer (avg), Da	MW HMW 1 (avg), Da	MW Multimer (avg), Da	MW LMW (avg), Da
pH 3 Control	145800	282800	1114000	114800
pH 3 T = 1 Day	145800	297500	836800	118900
pH 3 T = 3 Day	145800	304300	879700	114200

Sample ID/Lot	PD, Monomer (Mw/Mn)	PD, HMW 1 (Mw/Mn)	PD, Multimer (Mw/Mn)	PD, LMW (Mw/Mn)
pH 3 Control	1.000	1.000	1.018	1.061
pH 3 T = 1 Day	1.000	1.002	1.009	1.016
pH 3 T = 3 Day	1.000	1.009	1.012	1.004



- Acidic conditions display a large increase in multimeric species after 1 day
- Multimeric species show an array of dispersity with MW/Mn of 1.013, with sufficient separation

SEC-HPLC Chromatograms (A_{280}) of Cetuximab Subjected to **UV** Degradation Over 15 Hours

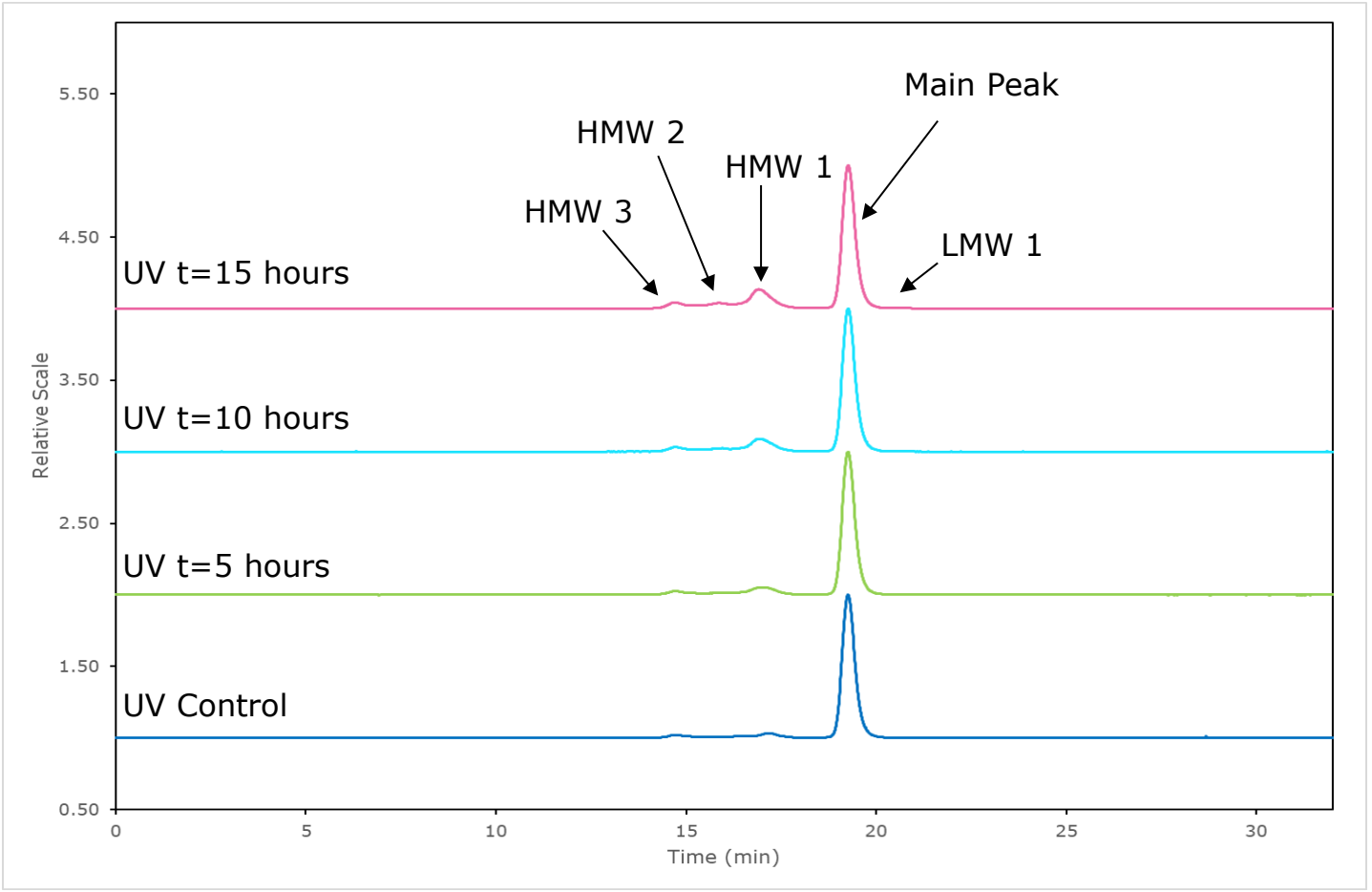


- Growth in HMW 1 over the time course, different than either thermal or acidic degradation
- Minimal, but apparent increase in fragments (LMW 1, LMW 2) after 10 hours of UV exposure

SEC-MALS Chromatograms & MW Data for Cetuximab Subjected to **UV** Degradation Over 15 Hours

Sample ID	MW Monomer (avg), Da	MW HMW 1 (avg), Da	MW HMW 2 (avg), Da	MW HMW 3 (avg), Da	MW LMW (avg), Da
UV Control	145800	285100	334500	1215000	118600
UV T = 5 hours	145800	287200	443400	1125000	118200
UV T = 10 hours	145800	288100	453800	1186000	116900
UV T= 15 hours	145800	286700	438500	1170000	113200

Sample ID	PD, Monomer (Mw/Mn)	PD, HMW 1 (Mw/Mn)	PD, HMW 2 (Mw/Mn)	PD, HMW 3 (Mw/Mn)	PD, LMW (Mw/Mn)
UV Control	1.000	1.001	1.006	1.017	1.035
UV T = 5 hours	1.000	1.000	1.001	1.030	1.024
UV T = 10 hours	1.000	1.000	1.052	1.032	1.024
UV T= 15 hours	1.000	1.000	1.001	1.017	1.013

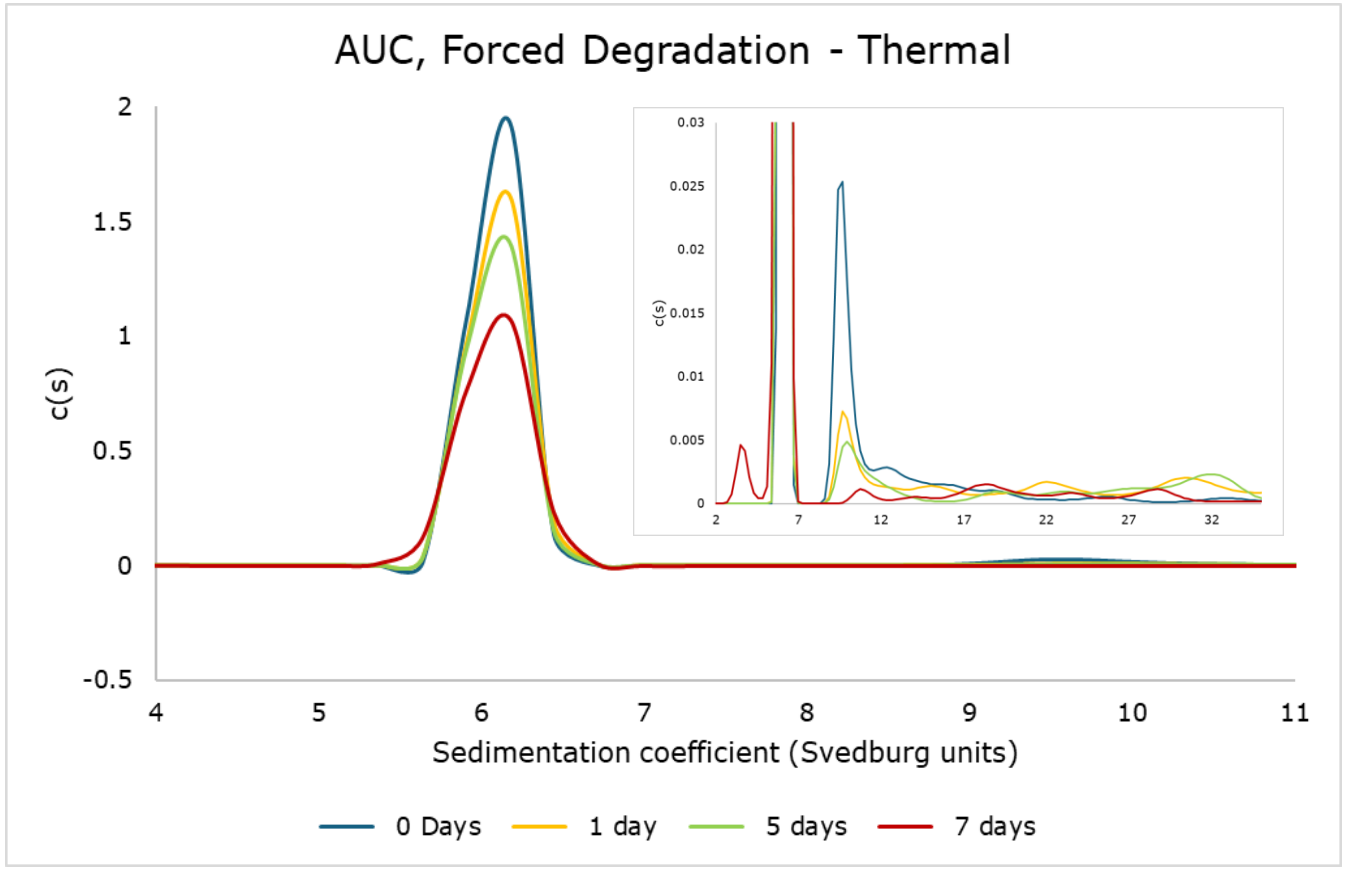


- Numerous HMW species seen in light scattering profile, with milder degradation than thermal or acidic conditions
- Dimeric, trimeric & large multimeric species apparent in light scattering with increasing PD

SV-AUC Analysis of Cetuximab Under **Thermal** Degradation at 50°C Over 7 Days

- Substantial level of degradation seen
- Decrease in main peak with corresponding increase in aggregation, with many HMW species >30 S

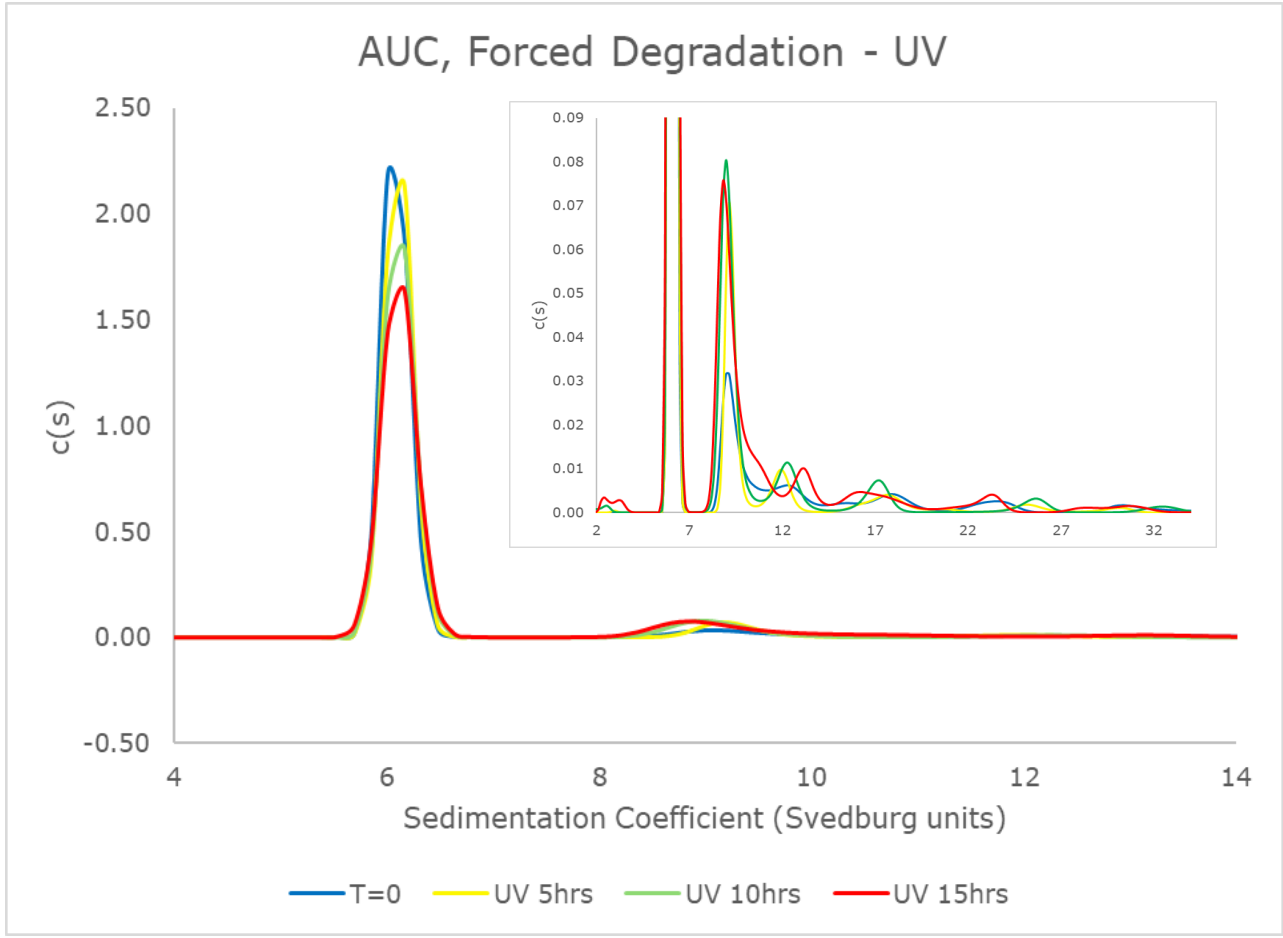
	Cetuximab, thermal degradation					
	Time (Days)	**Main Peak**	**HMW 1**	**HMW 2**	**HMW 3**	**HMW 4**
Sedimentation Coefficient (Svedberg Units, S, sec x 10^{-13})	**0**	**6.1**	9.8	13.4	19.9	25.7
	1	**6.1**	10.6	15.6	22.2	30.6
	5	**6.1**	10.9	19.2	23.0	26.6
	7	**6.1**	11.1	14.2	18.8	23.8
Molecular Weight (kDa)	**0**	**147**	301	478	867	1273
	1	**147**	334	598	1016	1649
	5	**146**	353	819	1075	1336
	7	**146**	360	520	792	1134
%distribution (*c*(*s*))	**0**	**94.2**	3.4	1.0	0.3	0.2
	1	**93.4**	1.6	0.6	1.1	1.4
	5	**86.8**	1.5	0.4	0.4	0.5
	7	**86.4**	0.3	0.2	1.0	0.4



SV-AUC Analysis of Cetuximab Under **Photolytic** Degradation (UV Exposure) Over 15 Hours

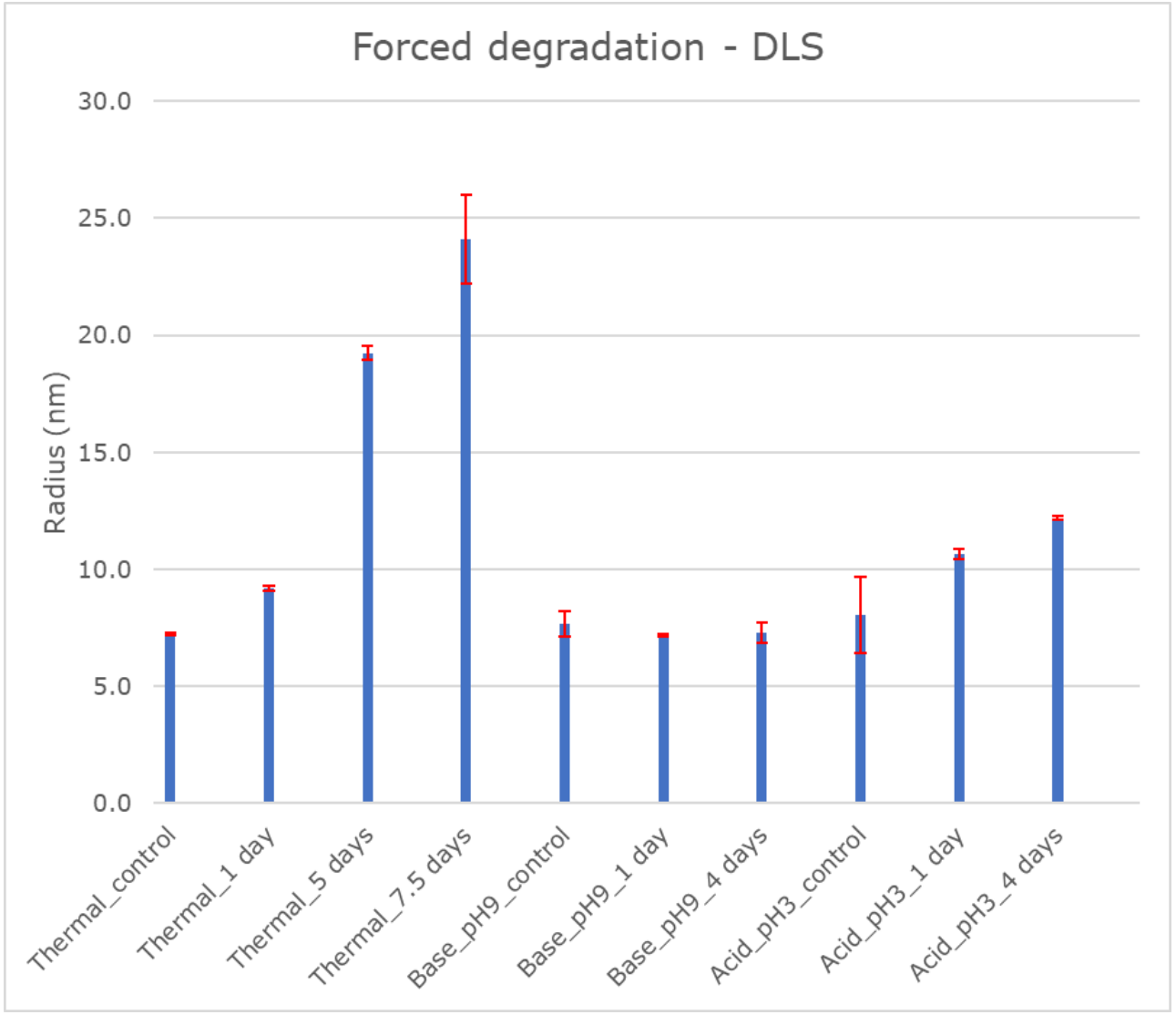
- Similar level of degradation seen in UV exposure as thermal at 50C
- Again, decrease in main peak with corresponding increase in aggregation
- UV is a potent degradant in mAb's

	Cetuximab, UV degradation					
	Time (Hours)	**Main Peak**	**HMW 1**	**HMW 2**	**HMW 3**	**HMW 4**
Sedimentation Coefficient (Svedberg Units, S, sec x 10^{-13})	**0**	**6.1**	9.5	12.5	17.9	NA
	5	**6.1**	9.3	12.0	17.5	NA
	10	**6.1**	9.2	12.2	17.0	25.3
	15	**6.1**	9.3	13.2	17.0	22.7
Molecular Weight (kDa)	**0**	**148**	295	446	768	NA
	5	**148**	282	413	731	NA
	10	**148**	277	432	705	1279
	15	**149**	288	481	702	1088
%distribution (*c*(*s*))	**0**	**92.5**	4.6	1.6	1.3	NA
	5	**91.7**	5.6	1.5	1.2	NA
	10	**86.9**	9.0	2.1	1.4	0.7
	15	**83.2**	11.8	1.8	1.9	1.0



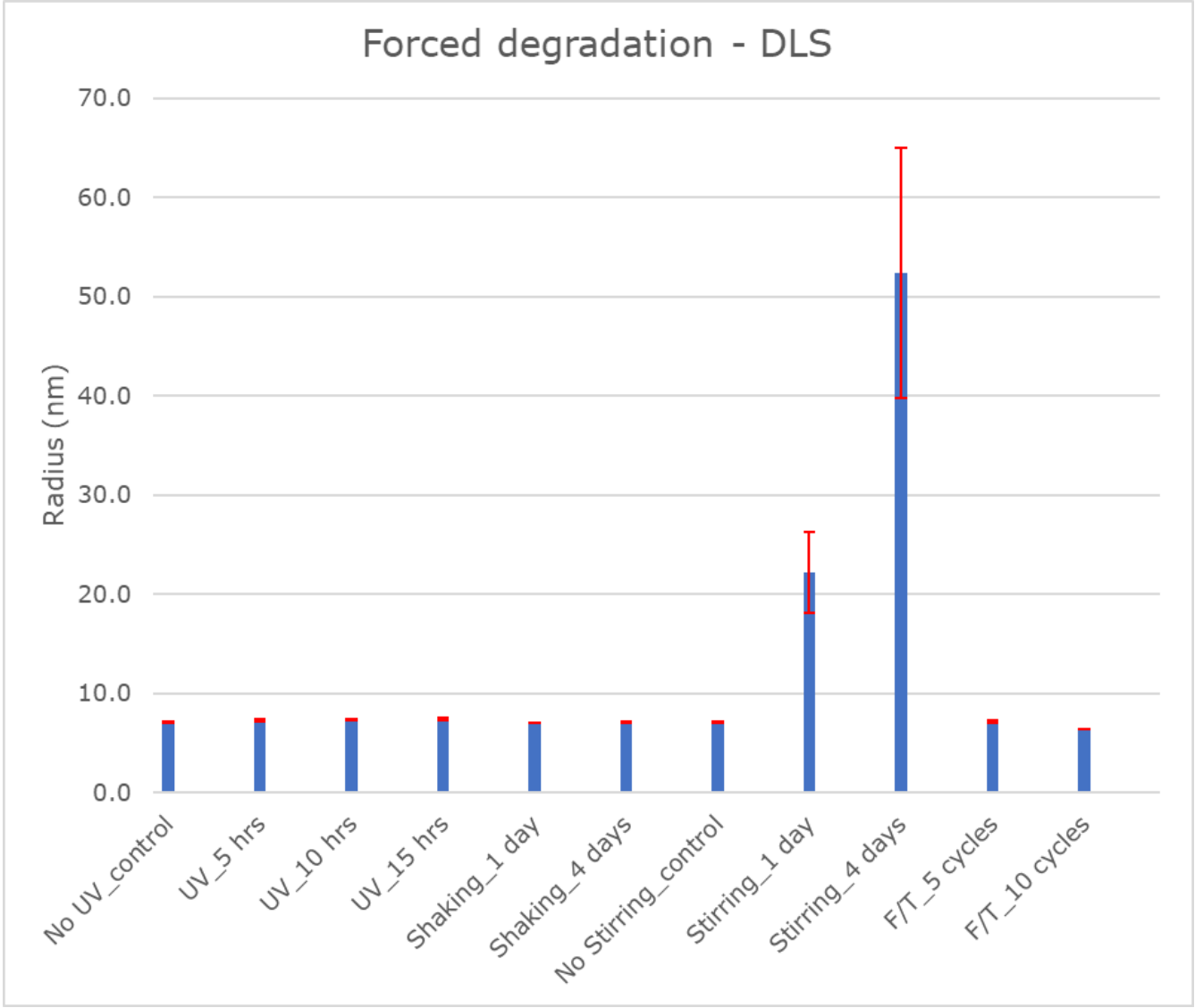
Dynamic Light Scattering Analysis of Cetuximab Under **Thermal, Basic** (pH 9) & **Acidic** (pH 3) Degradation Conditions

- Thermal degradation at 50C displays growth in overall size distribution of Cetuximab
- Base degradation (pH 9) is ineffective as a degradant
- Acidic degradation (pH 3) shows an increase in the radius, albeit in a slower fashion than thermal at 50C



Dynamic Light Scattering Analysis of Cetuximab Under **UV, Mechanical & Freeze/Thaw** Degradation Conditions

- Interestingly, UV, mechanical (shake), and freeze/thaw display little to no degradation over the time course
- Mechanical degradation by stirring (magnetic stir-bar) shows apparent degradation after 1 day and extreme increase in molecular radii at 4 days



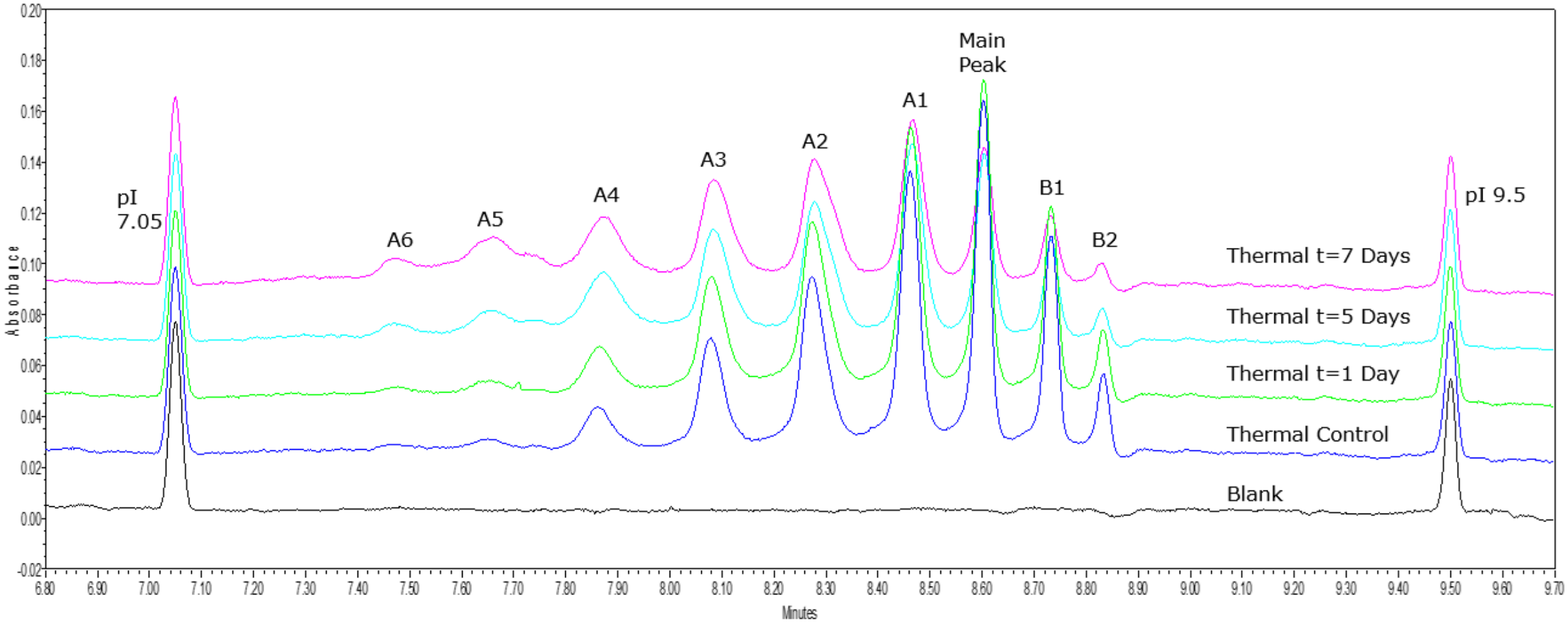
Dynamic Light Scattering (Results)

Measurements			
Sample	Radius (nm)	PDI	Diameter (nm)
No Stirring_control	7.1	0.068	14.1
Stirring_1 day	22.2	0.199	44.4
Stirring_4 days	52.4	0.374	104.8

- Stirring causes the most degradation in terms of overall aggregation of the molecule
- Stirring, thermal, and acidic conditions impart a moderate increase in polydispersity (0.1-0.4 PDI) on Cetuximab

Measurements			
Sample Temp	Radius (nm)	PDI	Diameter (nm)
Thermal_control	7.2	0.107	14.5
Thermal_1 day	9.2	0.247	18.4
Thermal_5 days	19.2	0.367	38.5
Thermal_7 days	24.1	0.339	48.2
Acid_pH3_control	8.0	0.285	16.1
Acid_pH3_1 day	10.7	0.176	21.3
Acid_pH3_4 days	12.2	0.296	24.4

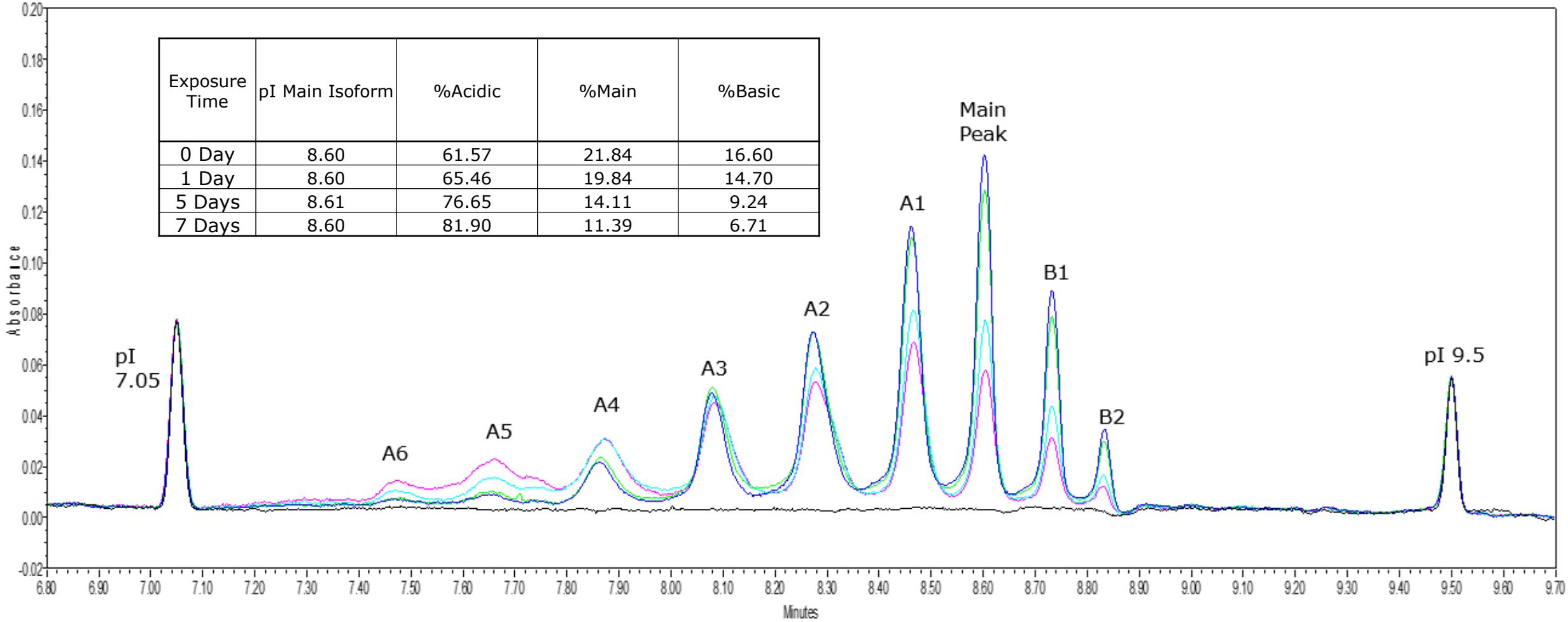
icIEF Analysis of Cetuximab Under **Thermal** Degradation at 50°C Over 7 Days



- Large increase in acidic variants seen with thermal degradation
- Corresponding area losses seen in main peak and basic variants

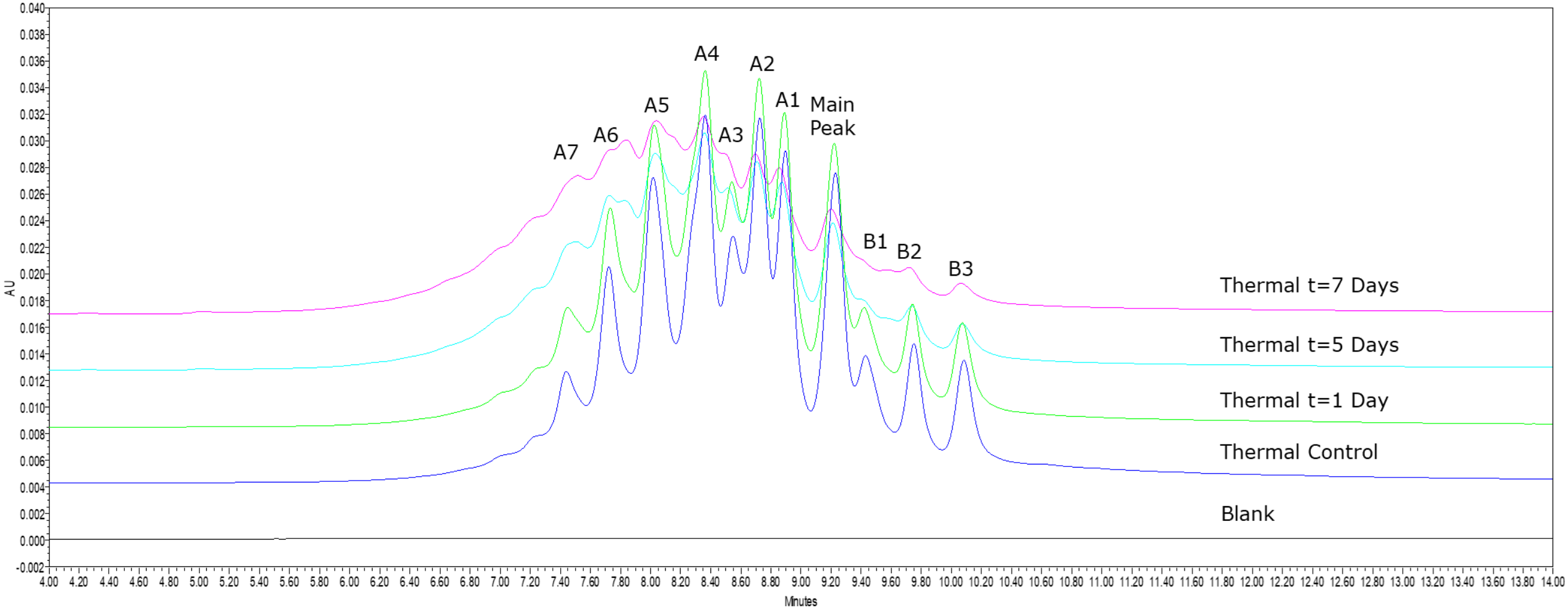
icIEF Analysis of Cetuximab Under **Thermal** Degradation at 50°C Over 7 Days (cont.)

Exposure Time	pI Main Isoform	%Acidic	%Main	%Basic
0 Day	8.60	61.57	21.84	16.60
1 Day	8.60	65.46	19.84	14.70
5 Days	8.61	76.65	14.11	9.24
7 Days	8.60	81.90	11.39	6.71



- ~33% increase in acidic variants at 7 days; ~24% at 5 days; ~6% at 1 day
- Conditions of ~2-3 days at 50°C produce ~10-15% loss in main peak (sensitive to heat)

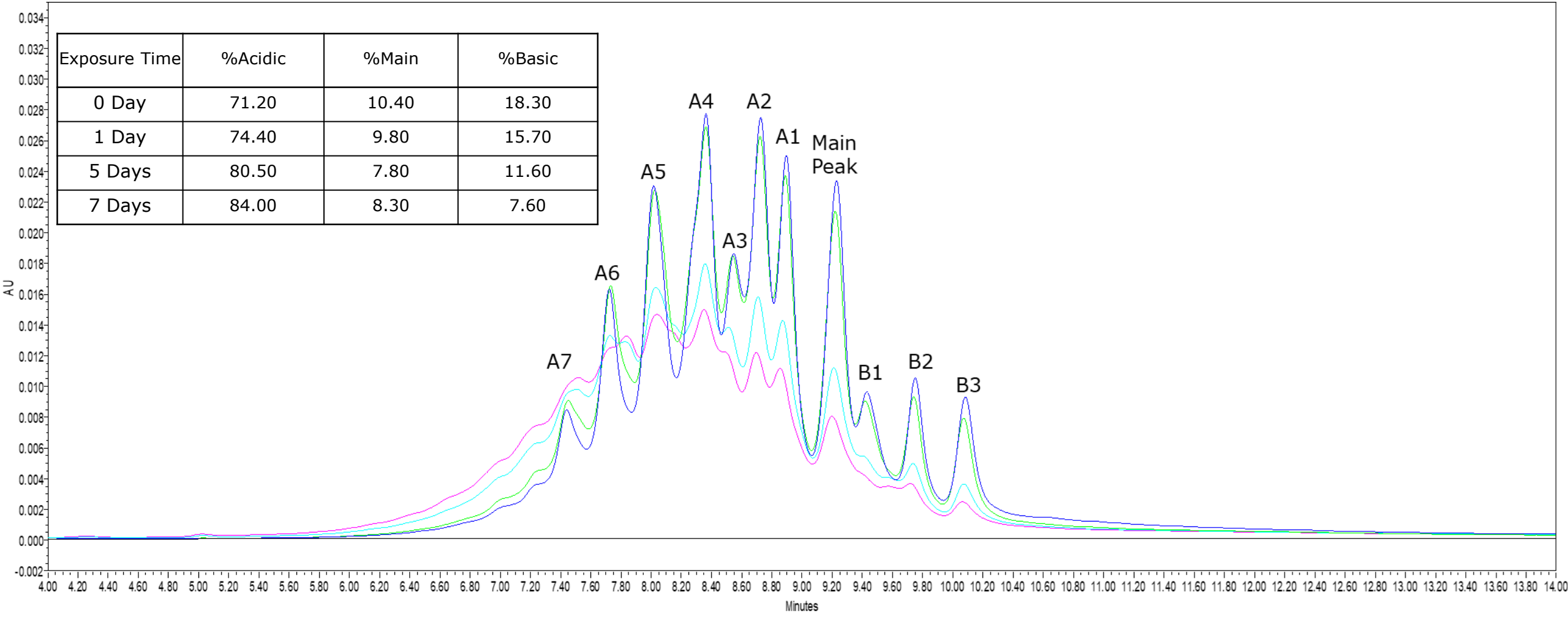
CEX Analysis of Cetuximab Under **Thermal** Degradation at 50°C Over 7 Day



- Large increase in acidic variants seen with thermal degradation, correlates with iCIEF

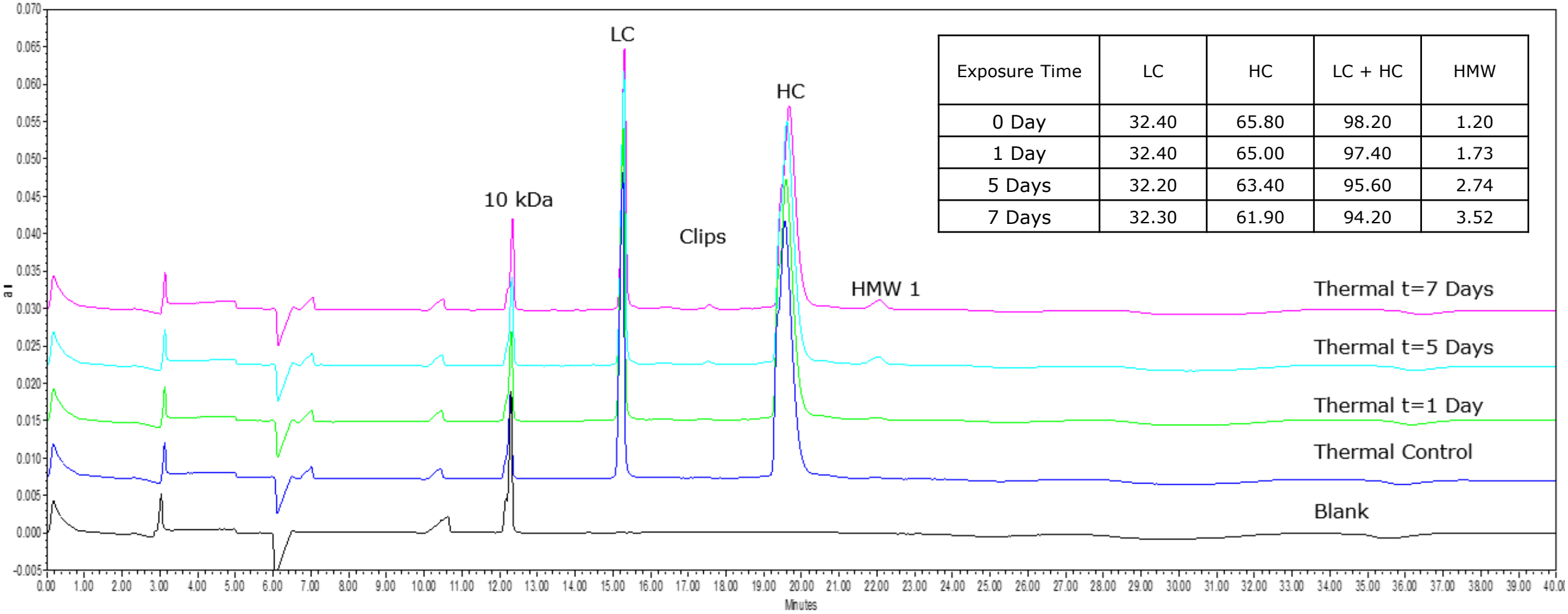
CEX Analysis of Cetuximab Under **Thermal** Degradation at 50°C Over 7 Day (cont.)

Exposure Time	%Acidic	%Main	%Basic
0 Day	71.20	10.40	18.30
1 Day	74.40	9.80	15.70
5 Days	80.50	7.80	11.60
7 Days	84.00	8.30	7.60



- Corresponding area losses seen in main peak and basic variants throughout time course

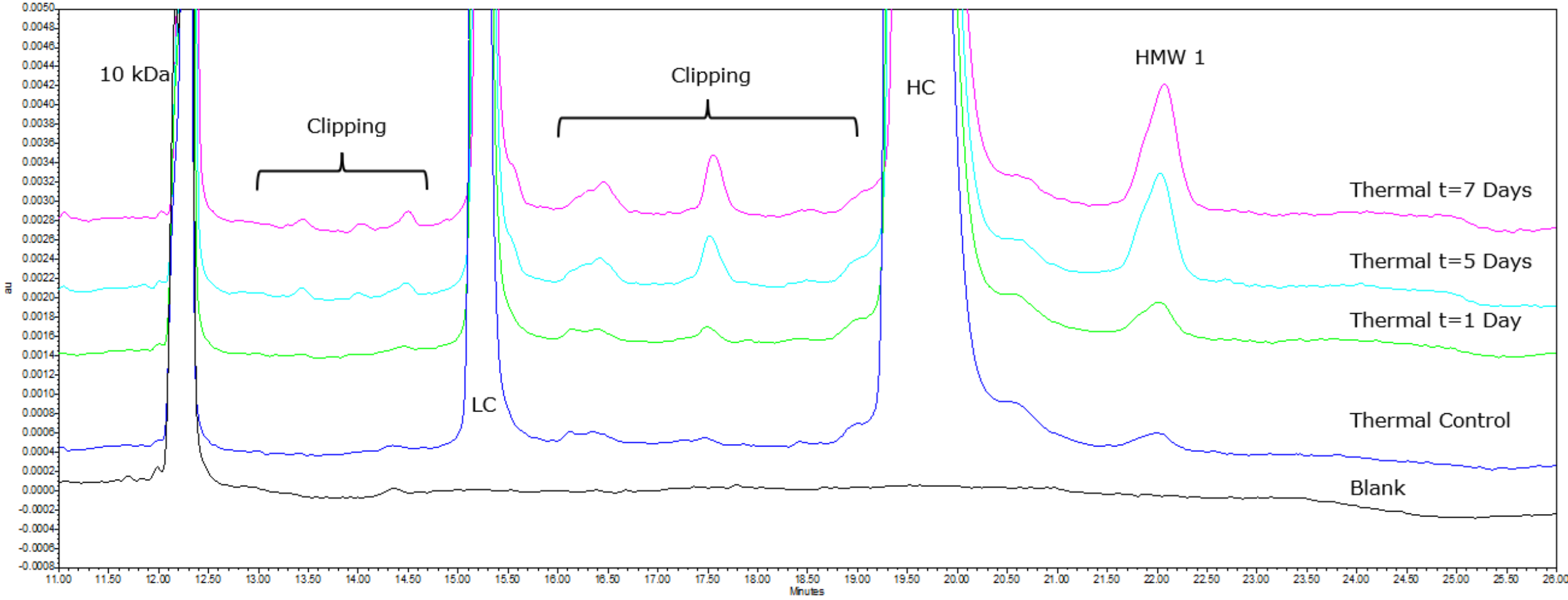
Reduced CE-SDS Analysis of Cetuximab Under Thermal Degradation at 50°C Over 7 Days



Exposure Time	LC	HC	LC + HC	HMW
0 Day	32.40	65.80	98.20	1.20
1 Day	32.40	65.00	97.40	1.73
5 Days	32.20	63.40	95.60	2.74
7 Days	32.30	61.90	94.20	3.52

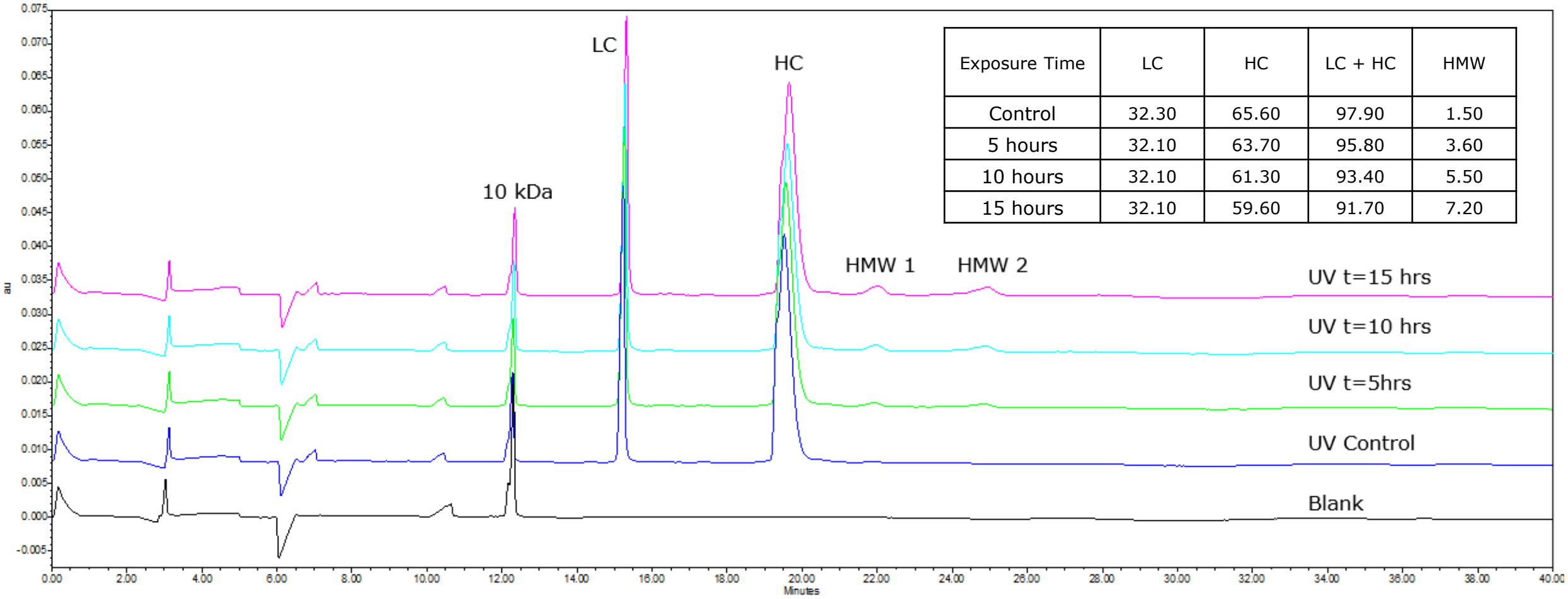
- Increase in HMW 1 over the time course, correlates with HMW 2 in SEC

Reduced CE-SDS Analysis of Cetuximab Under **Thermal** Degradation at 50°C Over 7 Days (cont.)



- Area losses in HC with corresponding increase in HMW; LC remains consistent throughout study

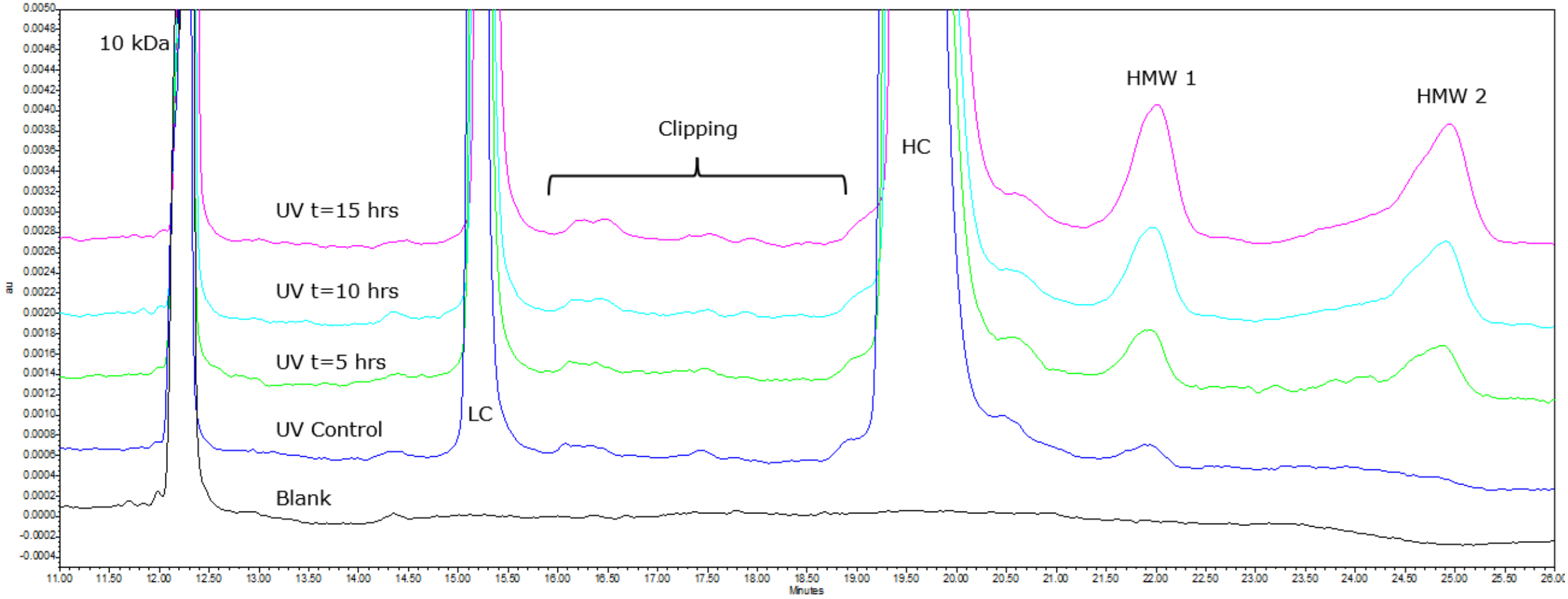
Reduced CE-SDS Analysis of Cetuximab Under **UV** Degradation Over 15 Hours



Exposure Time	LC	HC	LC + HC	HMW
Control	32.30	65.60	97.90	1.50
5 hours	32.10	63.70	95.80	3.60
10 hours	32.10	61.30	93.40	5.50
15 hours	32.10	59.60	91.70	7.20

- Increase in HMW 1 along with HMW 2 species growth caused by UV exposure after 5 hours

Reduced CE-SDS Analysis of Cetuximab Under **UV** Degradation Over 15 Hours (cont.)



- Different degradation pathway seen in UV exposure as compared to thermal at 50C with HMW 2 species resolution

Forced Degradation Study Summary

Essential Insights Into the Stability of Cetuximab

1. Thermal & UV exposure led to significant degradation in aggregation assays (SEC, SV-AUC, CE-SDS)
2. Early aggregation is observed with dimeric and high MW species at 10-30% area
3. Acidic degradation results in numerous aggregates, as confirmed by SEC & SV-AUC
4. Charge-based assays (CEX & icIEF) show similar results, indicating orthogonality
5. Consistent correlation is noted between SEC, SV-AUC & CE-SDS
6. Magnetic stirring increases molecule diameter in DLS

Strategic Role of FD Studies in IND Applications & LCM

Manufacturability

- Optimize formulation & storage conditions
- Remove vulnerabilities such as aggregation or degradation triggers

Critical Quality Attributes (CQAs)

- Assess molecule's structural integrity & functional activity
- Monitor to ensure product safety & efficacy

Method Development & Validation

- Establish stability-indicating methods with full/limited FD panel assessments
- Validate & qualify methods for reliable performance

Lot-to-Lot Comparability

- Evaluate consistency through limited FD panel testing
- Detect variations early & maintain product quality

Early & consistent integration of forced degradation studies ensures robust method development, successful IND submissions & lifecycle management (LCM) of your molecule

AGENDA

1. Why Forced Degradation Matters
2. Major Degradation Pathways
3. Forced Deg Study: Cetuximab
4. Execution: Forced Degradation Results
5. **Your Molecule at Catalent**
6. Q&A

What is Right for Your mAb, ADC, Fusion Protein?

Monitoring Critical Quality Attributes through QC-friendly methodologies

Catalent offers all the aforementioned technology plus

- LC-MS, chromatography, electrophoresis, cell-based and bioassay, etc.
- Full-scale stability offerings

Biophysical/Biochemical/Bioassay

- Approximately 200+ scientists
- Dedicated BioAnalytics technical lead and QA team
- 30+ years of providing exceptional customer experience

Discovering stability issues early means fewer surprises later

Catalent®
BIOLOGICS

Q&A

Follow us on **LinkedIn** to learn about new offerings and the latest and news!

SCHEDULE A MEETING FOR TAILORED DISCUSSIONS:

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