Challenges in Product Specifications with Asian Regulatory Authorities: A Case Study

Auquier Vanessa, IABS Tokyo, 23-25 June 2025

Inspired by **patients.**
Driven by **science.**

Agenda

1. Introduction on size variants – specification and analysis
2. Literature input
3. Challenges from the Health Authorities
4. Conclusions

Specifications for mAbs – size variants

- Specifications for monoclonal antibodies (mAbs) should be designed to ensure ***quality, safety and efficacy of the drug product***
- Various antibody variants have to be assessed during product development and at commercial stage
- The ***Critical Quality attributes (CQA)*** will have to be controlled to ensure patient safety and product efficacy.
- ***Size variants*** are typical species assessed and presenting important CQA

Monomer
(intact mAb HLHL, ~150kDa)

High Molecular Weight Species (HMWS)
(mainly dimers and aggregates up to 2µm in size)

Low Molecular Weight Species (LMWS)
(fragmentation of the intact mAb)
(eg HHL, HL, LL, L, Fab' etc)

- HMWS have typically a high to very high impact on biological activity, PK/PD, immunogenicity and safety → CQA and therefore controlled via the product specifications.
- LMWS might be critical to assess ***manufacturing and product stability*** consistency and could have an impact on biological activity and PK/PD. In this case the LMWS will be a CQA and would need to be controlled via the product specifications.
- Monomer or purity is a quality and regulatory expectation hence will be monitored through the product specifications.

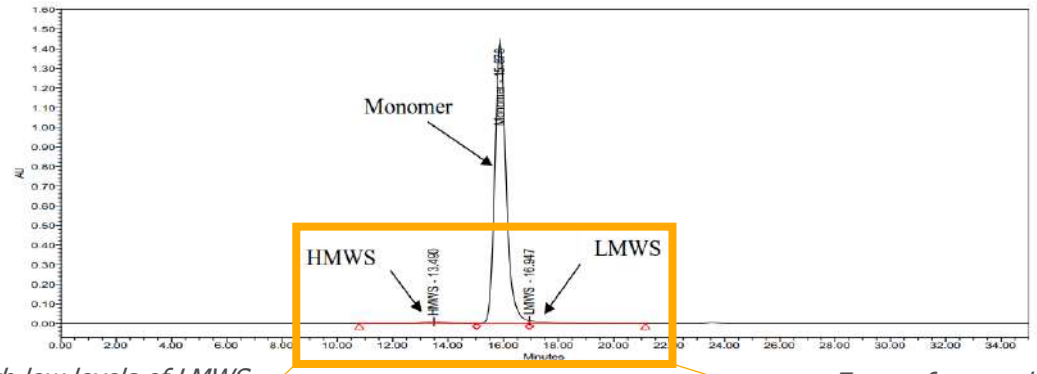
Size variants – Size Exclusion

- Two main techniques used to analyse the size variants for mAbs:

  Size Exclusion chromatography (SEC) and ***Non-Reducing Capillary Gel Electrophoresis (NR-CGE)***

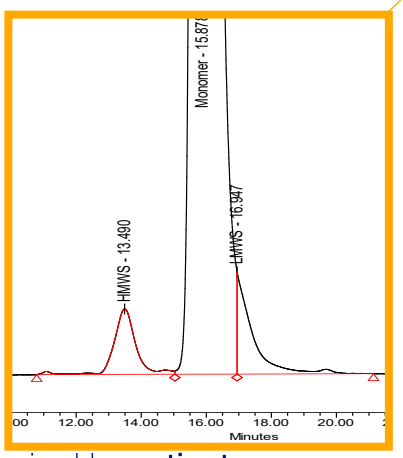
- **SEC** is recognized as the preferred technic for ***High Molecular Weight Species (HMWS)***

Typical SEC profile

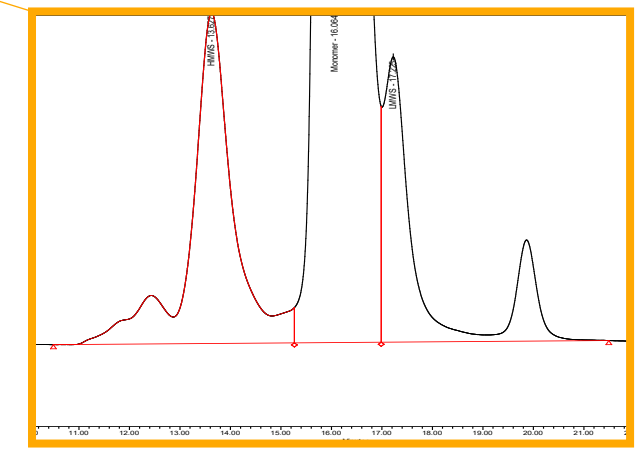


No appropriate separation of the LMWS and Monomer

Zoom of a sample with low levels of LMWS



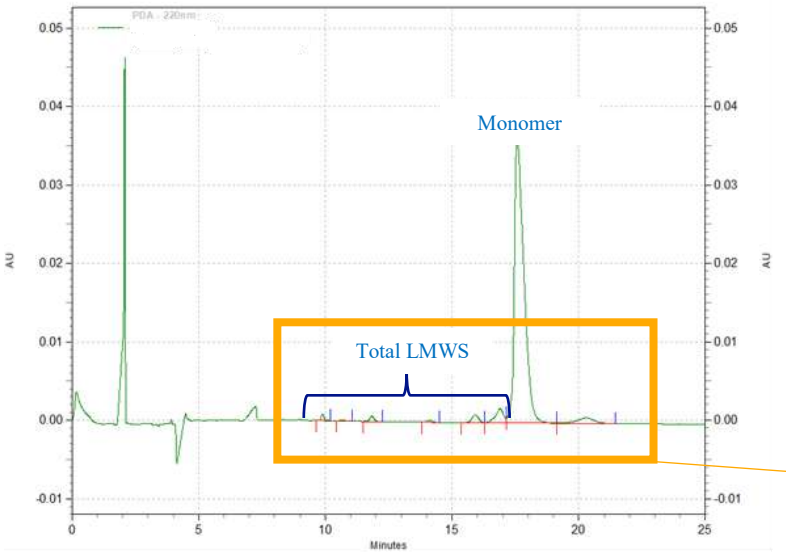
Zoom of a sample with high levels of LMWS



Size variants – Non-Reducing Capillary Gel Electrophoresis

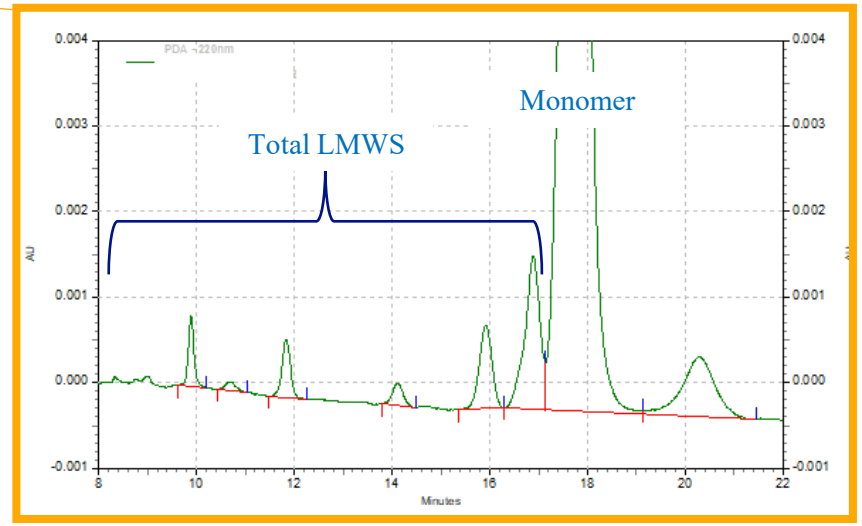
- **NR-CGE** is known as a more reliable method for the quantification of ***LMWS*** in denaturing conditions

Typical NR-CGE profile



Accurate quantification of the ***Low Molecular Weight Species (LMWS) and Monomer***

Zoom of a sample with high levels of LMWS

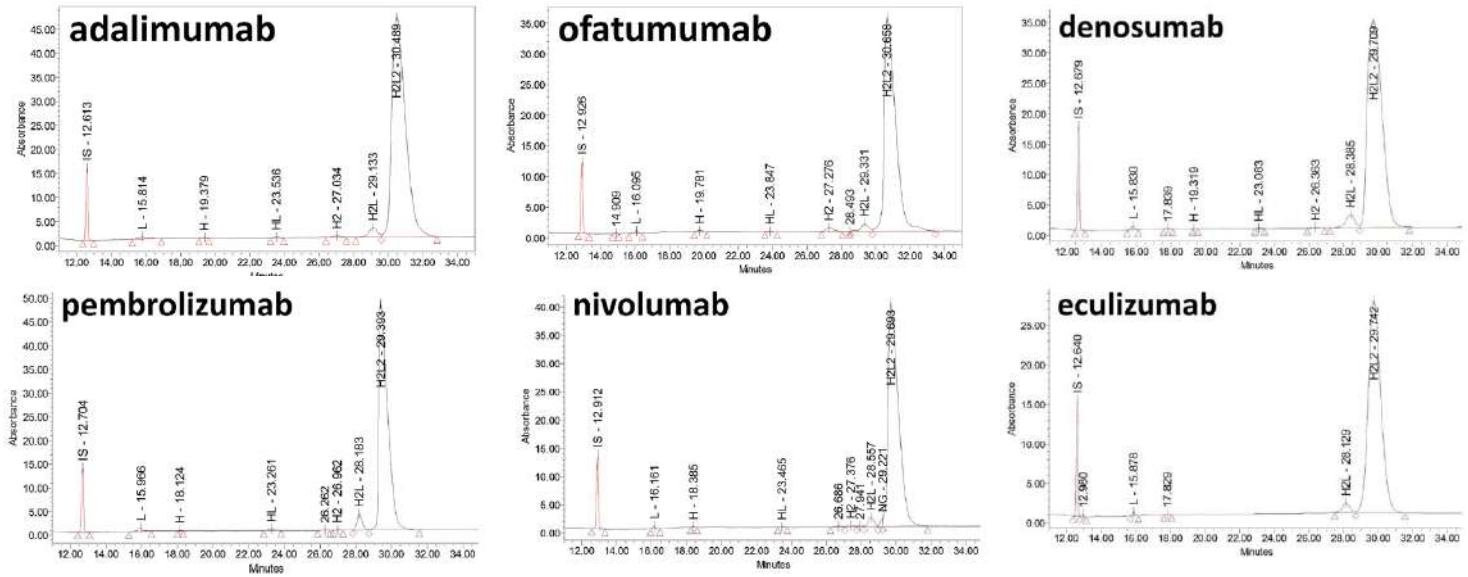


Not all HMWS could be detected by NR-CGE (typically only dimers)

Size variants analyses for mAbs – literature

- According to ***USP monograph 129***, SEC is considered a robust method for the HMWS determination while NR-CGE is more reliable for the LMWS quantification.
- NR-CGE has become over the last years a ***key method in Quality Control laboratories*** for size-variants analysis of antibodies
- This is also confirmed by a ***recent article from 2020 of Wagner & al.****:

  A generic NR-CGE method was tested for a wide range of ***26 FDA and EMA approved mAbs and 2 antibody drug conjugates (ADCs) as well as for the NISTmab***, in a QC environment.



<u>Conclusion:</u> NR-CGE is a state-of-the-art analytical technique, scientifically recognized to be more powerful, sensitive and robust for the determination of monomer and LMWS.

**Wagner, E. et al, Journal of Pharmaceutical and Biomedical Analysis Volume 184, 30 May 2020, 113166: Determination of size variants by CE-SDS for approved therapeutic antibodies: Key implications of subclasses and light chain specificities*

Specifications for mAbs – size variants

If the size variants are identified as ***Critical Quality attributes (CQA)*** they will have to be controlled through the product specifications with the most appropriate method for their quantification.

- HMWS will be controlled via the SEC method.
- LMWS will be controlled via the NR-CGE.
- Monomer will be controlled via the NR-CGE.

Note: during product development the monomer and/or LMWS can be also monitored via the SEC to support product knowledge but at time of commercial only the most appropriate quantification should be put in place to ensure accurate and robust control of the species.

If a specification is set up for each of these species even if via different methods, most of the Health Authorities will accept this strategy.

Size variants – Challenges from Health Authorities

Round 1:

- Some Health Authorities, mainly in Asian countries (as South Korea or China), still consider that the monomer should be also monitored by the SEC method even if the monomer determination by NR-CGE is more appropriate.
- Purity and impurities should be tested using each test, NR-CGE and SEC according to these authorities.

Push back:

- Beside all previous explanations and literature support, we provided a demonstration of statistical correlation for monomer between the 2 methods.
- The strong correlation obtained for monomer between the two methods provides assurance that the purity measured by NR-CGE is ***representative*** of the purity by SEC but without the bias introduced because of the lack of baseline resolution between monomer and LMWS in the SEC method
- Bridging study between monomer levels measured by NR-CGE and those measured by SEC was done with the following statistical tools:

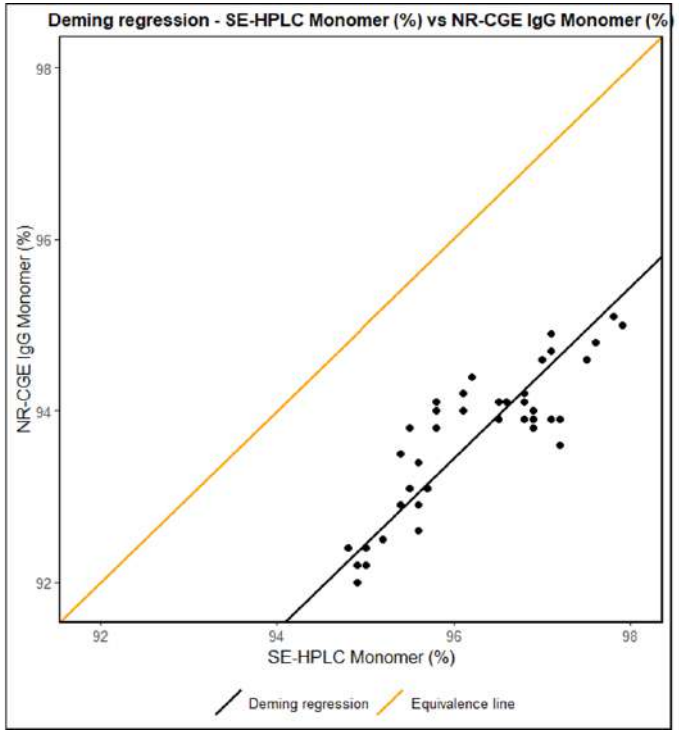
1. *Pearson correlation coefficient*, which is used to understand the degree of linear correlation between two attributes.
2. *Deming regression*, which is used to directly model the results of one attribute as a function of the results of the other attribute.
3. *Bland-Altman approach*, which is used to model the bias between two attributes as a function of their average result.

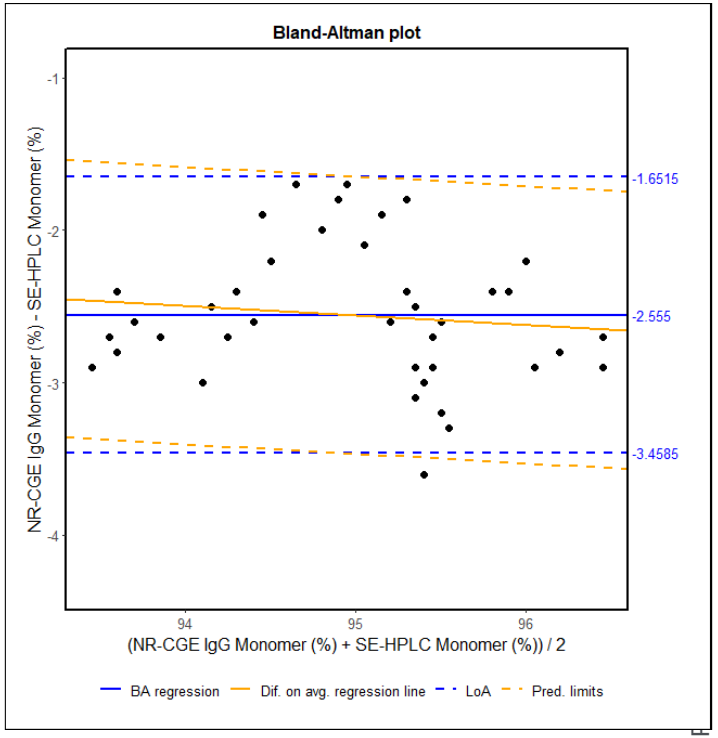
Size variants – Challenges from Health Authorities

→ Bridging results:

1. *The Pearson correlation* coefficient shows a very strong correlation between the two analytical methods with a correlation coefficient of 0.86.
2. *The Deming regression* analysis demonstrates visually a constant bias between the two methods (bias of 2.55% based on intercept).
3. *The Bland-Altman analysis* shows that the bias between the two methods is statistically significant and constant, with an estimate of approximately 2.56%.

Therefore, the monomer levels measured by the NR-CGE method are consistently ~2.6% lower than the monomer levels measured by SEC method.





Conclusion: monomer determination by SEC is not appropriate and not required as there is a strong correlation with the NR-CGE measurement which is more accurate due to the inherent method performance. Due to the better separation by NR-CGE, the purity determination is worst case by NR-CGE vs SEC.

Size variants – Challenges from Health Authorities

Round 2:

- Still not accepted by the HA, the SEC was used still in phase 3 and the NR-CGE only introduced during phase 3 so there is limited commercial experience.
- For the HA, all registered recombinant pharmaceuticals report monomer using SEC (ie HA expectation).

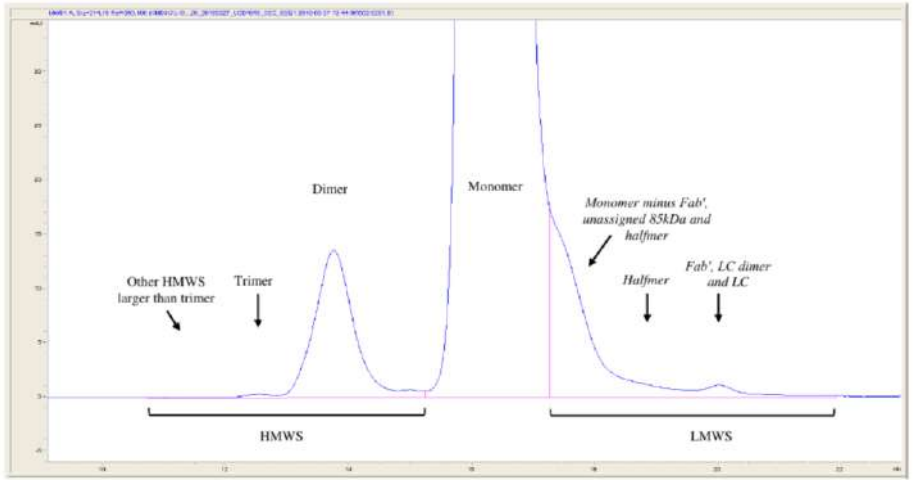
Push back:

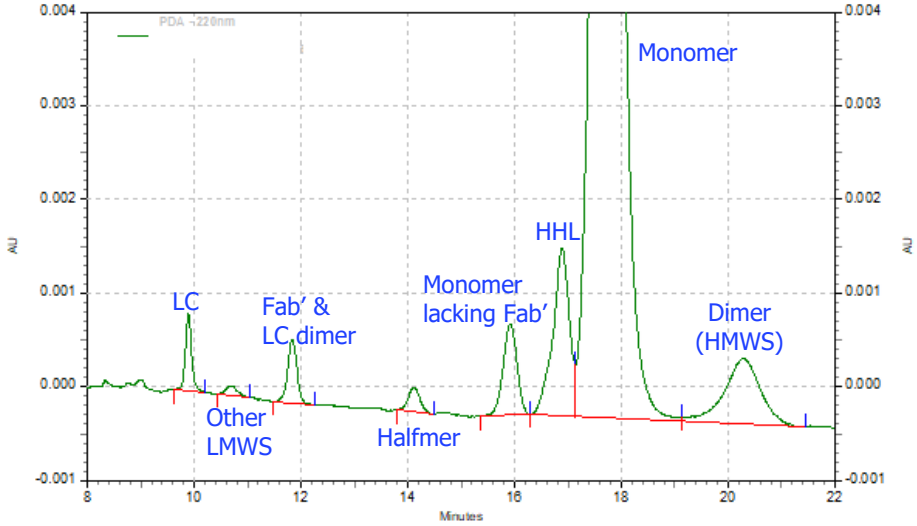
- The control strategy, incl specification limits, has integrated the knowledge gathered during the development of the product.
- Product is approved in 16 countries worldwide using the control strategy and specifications which represents a large set of patients and therefore supports the appropriateness for maintaining product quality and ensuring patient safety.
- Characterisation data of the SEC method: ***heavy chain – heavy chain – light chain (HHL)*** fragment is co-eluting in the tailing edge of the monomer peak.
- Separation of such small difference in size (~25kDa) between the HHL (~125kDa) and the monomer (~150kDa) can not be achieved by SEC as the size is the separation principle. While the NR-CGE could achieve a valley separation of the species.
- This difference in the HHL separation from the monomer between the 2 methods is confirmed by the bridging study as the bias of ~2.6% corresponds predominately to the HHL levels resolved by the NR-CGE method.
- Stability data illustrate this assessment.

Size variants – Challenges from Health Authorities

→ Characterisation, method bridging and stability data

- During phase 3, the SEC characterisation showed that the HHL is co-eluting with the monomer → need for an improved method, hence the NR-CGE was put in place.





- Bridging study between both methods : bias of ~2.6% (monomer by NR-CGE is ~2.6% lower than by SEC)
- Stability data:

Stability timepoint	SEC data (%)		NR-CGE data (%)	
	LMWS	Monomer	LMWS	Monomer
Release	1.1	97.6	4.2	94.8
12 months	1.2	96.7	4.5	94.1
24 months	1.4	96.1	4.9	93.5

Size variants – Challenges from Health Authorities

Round 3:

- Still not accepted by the HA.
- Request to demonstrate that no better resolution could be achieved by SEC

Follow up:

- As requested by the HA a specification has been put in place for the monomer by SEC.
- Further SEC method development was conducted to evaluate alternative column and chromatographic conditions
- More ***recent state of the art SEC columns*** and ***different experimental conditions*** (eg variations of mobile phase pH and salt) were assessed to obtain better baseline separation between monomer and LMWS
- Although more recent and state of the art SEC columns were evaluated with different conditions, no baseline separation between monomer and LMWS was obtained.
- No major improvement was achieved as expected due to the closeness in size of the monomer species and first eluted LMWS (HHL). Separation of close masses is too challenging for SEC.

Conclusion

- Despite strong scientific arguments based on method performance, statistical analysis and characterization data
- Despite literature evidence
- Although 16 countries had already accepted

→ We had to implement ***additional specifications for the monomer by SEC*** to avoid delaying patient access in these Asian countries.

Is there a way to revise such old standard approaches and HA expectations when good rationales are available?

Aim : harmonizing specifications across regions to improve product quality but also expedite patient access to essential medications.

Similar cases exist with all HA across the world for various parameters which represents huge challenges for the industry:

- Example: Request for setting specifications for the charge variants which are not identified as CQA (charge variants are good to ensure manufacturing consistency but have no impact on patient safety and product efficacy)

Thanks to team involved in this multidisciplinary exercise!

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