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Finnish Medicines Agency

Establishment of robust compendial methods for analysis of monoclonal antibody products

CMC Strategy Forum Europe 2025

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Disclaimer

The views presented are my personal opinions and should not be taken as official guidelines of the Finnish Medicines Agency or EDQM

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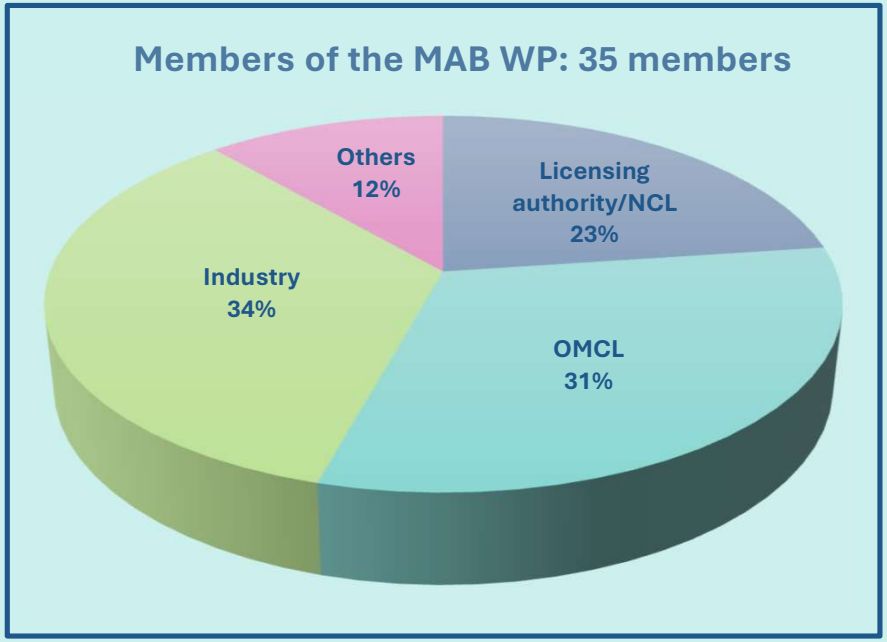
MAB Working Party

Horizontal standards

- 2.7.26 Cell-based assays for potency determination of TNF-alpha antagonists
- 2.5.44 Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies
- **2.5.43 Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies**



SEC Collaborative study

- **Two SEC procedures:** HPLC and UHPLC
- **A two-arm study:**
  - Arm 1: regulators (10 labs, 6 mAbs)
  - Arm 2: industry (8 labs, 16 mAbs)
- **Purpose of the study:**
  - Show applicability for **quantification of the monomer and HMWS** of multiple mAbs
  - **Validate the procedures**
  - Provide data to support setting system performance and SST criteria

Study setup

- Common study protocol
  - SEC-HPLC (Procedure A)
  - SEC-UHPLC (Procedure B)
- Common reference standards
  - Molecular weight standard
  - mAb CRS
  - In-house standard
- Centralised data evaluation (27 data sets)
- Performance characteristics: accuracy, precision, reporting threshold, and linearity over the reportable range (low range for HMWS and target range for main peak)

SAMPLE INFORMATION

Sample Name:	Ref sol (b) MW	Acquired By:	Samaletdin
Sample Type:	Standard	Sample Set Name:	240425_samples
Vial:	1:A,2	Acq. Method Set:	240423HS
Injection #:	2	Processing Method:	240514_MW
Injection Volume:	2.50 ul	Channel Name:	280.0nm
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	PDA 280.0 nm (PDA Spect (210-400)nm)
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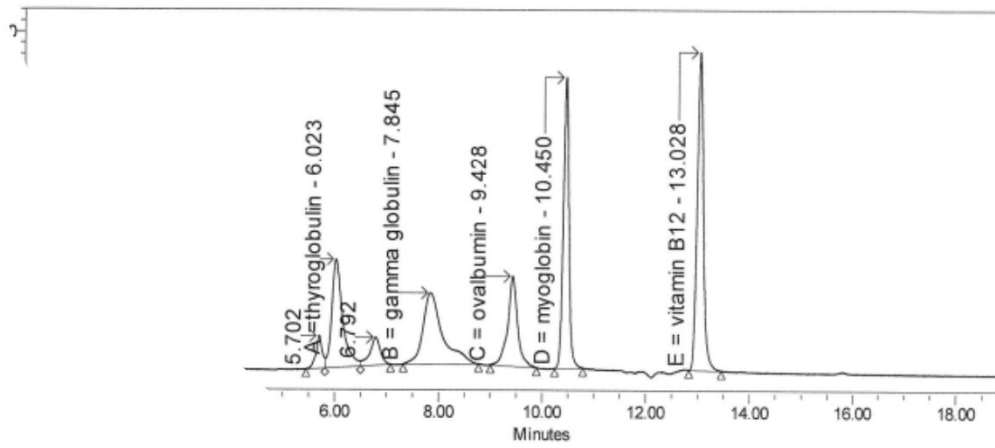

RT	Area	% Area	Height	Resolution
	23546	2.59	2915	
		15.59	9679	1.2
			2492	2.3
				2.3

The three levels of reference standards

System performance:
Molecular weight standard

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Sample Name:	Ref sol (b) MW	Acquired By:	Samaletdin
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Vial:	1:A,2	Acq. Method Set:	240423HS
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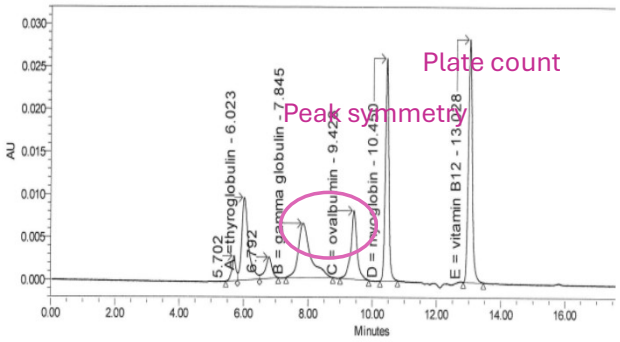

	Peak Name	RT	Area	% Area	Height	Resolution
1		5.702	23546	2.59	2915	
2	A =thyroglobulin	6.023	141816	15.59	9679	1.2
3		6.792	34868	3.83	2492	2.3
4	B = gamma globulin	7.845	167688	18.44	6367	2.3
5	C = ovalbumin	9.428	108235	11.90	8030	3.6
6	D = myoglobin	10.450	194173	21.35	26061	4.2
7	E = vitamin B12	13.028	239064	26.29	28469	12.9

System suitability:
mAb CRS

SAMPLE INFORMATION

Sample Name:	Ref sol (a)	Acquired By:	Samaletdin
Sample Type:	Standard	Sample Set Name:	240426_SST_Ref_s
Vial:	1:F,5	Acq. Method Set:	240423HS
Injection #:	1	Processing Method:	240530_ref a
Injection Volume:	2.50 ul	Channel Name:	280.0nm
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	PDA 280.0 nm (PDA (210-400)nm)
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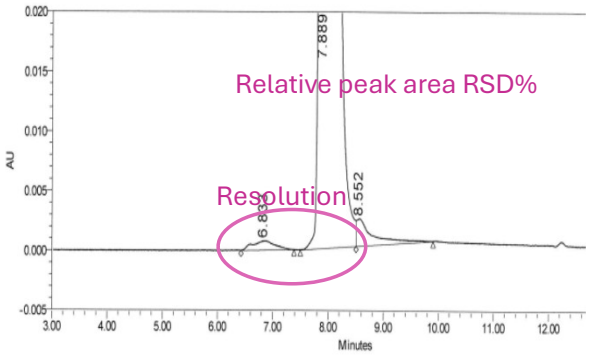

	RT	Area	% Area	Height	Resolution	Symmetry Factor
1	6.833	22146	0.82	796		1.07
2	7.889	2635953	97.32	168994	1.7	2.41
3	8.552	50305	1.86	2302		

	EP Plate Count
1	1068
2	5942

Sample acceptance criteria:
in-house standard

SAMPLE INFORMATION

Sample Name:	Ref std	Acquired By:	Samaletdin
Sample Type:	Standard	Sample Set Name:	240425_samples
Vial:	1:C,7	Acq. Method Set:	240423HS
Injection #:	2	Processing Method:	240429HS
Injection Volume:	2.50 ul	Channel Name:	280.0nm
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	PDA 280.0 nm (PDA (210-400)nm)
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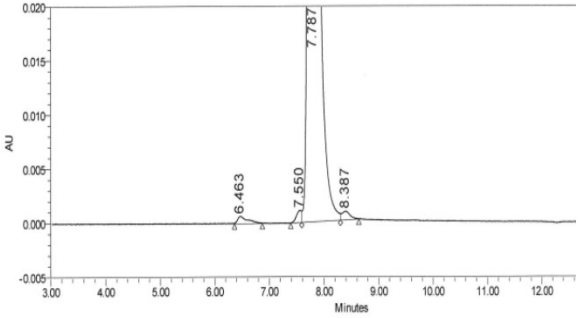
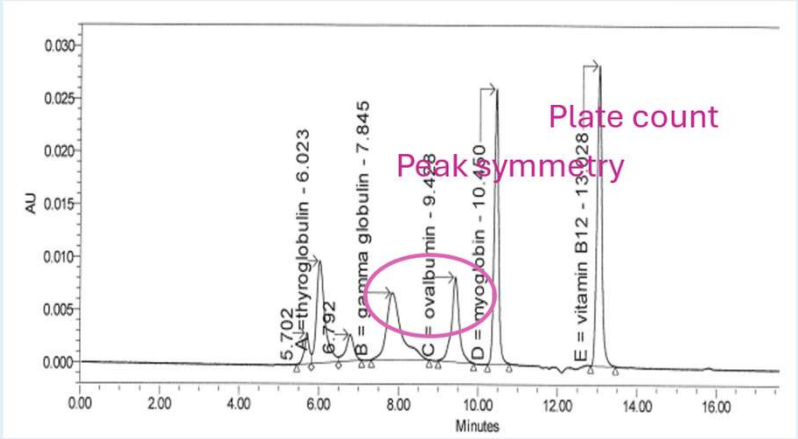


	RT	Area	% Area	Height	Symmetry Factor	EP Plate Count
1	6.463	8814	0.36	688	2.45	9578
2	7.550	6980	0.28	1120		
3	7.787	2455187	99.03	291327	1.30	21809
4	8.387	8374	0.34	803		

System performance: Molecular weight marker



- The system performance criteria are derived from the statistical analysis of the results
- The criteria are the same but the requirements are different for the HPLC and UHPLC procedure

SEC-HPLC (Procedure A)	SEC-UHPLC (Procedure B)
• All five peaks visible • Resolution: min 1.8 between ɣ-Globulin /Ovalbumin • Theoretical plates: min 13 000 for B 12 peak • Symmetry factor: 0.9-1.3 for Myoglobin peak • Repeatability: max 2% RSD for B12 peak area (n=3)	• All five peaks visible • Resolution: min 2.5 between ɣ-Globulin /Ovalbumin • Theoretical plates: min 38 000 for B12 peak • Symmetry factor: 0.9-1.5 for Myoglobin peak • Repeatability: max 2% RSD for B12 peak area (n=3)

2.5.43: System performance is assessed periodically using the molecular weight standard

System suitability: Monoclonal antibody for SEC system suitability CRS

SAMPLE INFORMATION

Sample Name:	Ref sol (a)	Acquired By:	Samaletdin
Sample Type:	Standard	Sample Set Name:	240426_SST_Ref_s
Vial:	1:F,5	Acq. Method Set:	240423HS
Injection #:	1	Processing Method:	240530_ref a
Injection Volume:	2.50 ul	Channel Name:	280.0nm
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	PDA 280.0 nm (PDA (210-400)nm)
Date Acquired:	4/26/2024 5:35:14 PM EEST		
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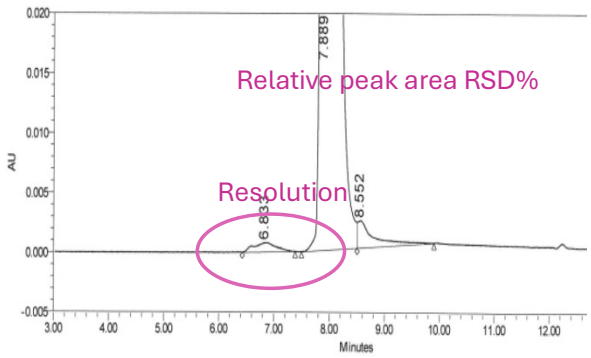
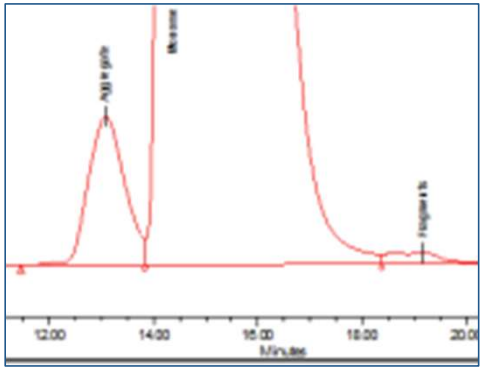


	RT	Area	% Area	Height	Resolution	Symmetry Factor
1	6.833	22146	0.82	796		1.07
2	7.889	2635953	97.32	168994	1.7	2.41
3	8.552	50305	1.86	2302		

	EP Plate Count
1	1068
2	5942

SST criteria for both procedures

- No interfering peaks observed in the blank solution
- Visibly similar to the chromatogram in the CRS leaflet
- Resolution: the monomer and the preceding peak are separated as in the leaflet
- Repeatability: max 1% RSD for the relative peak area of the monomer (n=3)



Validation using 19 mAbs

Specificity

Range

linear response at lower (HMWS) and target (monomer) range

Precision

Accuracy

Collaborative study, Summary

- The SEC procedures have been validated according to accepted scientific principles for the quantitation of HMWS and the monomer using 19 different mAbs
    - specificity, reporting threshold, linearity, accuracy, and precision
- The SEC procedures are robust, as shown by pooling the results from 18 different laboratories
- Altogether 27 data sets were submitted and analysed
- The procedures may be used for monitoring the quality and production consistency of a mAb

2.5.43 Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies - outline

- Introduction and scope
- Procedures
  - Procedure A (HPLC)
    - System performance
    - System suitability
    - Sample acceptance criteria
  - Procedure B (UHPLC)
    - System performance
    - System suitability
    - Sample acceptance criteria
- Data analysis
  - Instructions for integration
- Results
- General recommendations
  - Practical considerations
    - Understanding sample stability / behavior
    - Use of control samples
    - Continued performance to be controlled
  - Points to consider in validation required for a specific mAb
    - Extent of validation is determined based on the method's suitability and justified to the satisfaction of competent authority
    - Examples requiring validation: different peak grouping/integration
- Validation
  - To show suitability for a mAb: assessment of applicability is necessary to evaluate the extent of validation studies
  - If used as is, verification studies according to *5.26. Implementation of pharmacopoeial procedures* is sufficient

→ Published in Pharmeuropa 1/2025 for comments
→ To be presented for adoption by Ph Eur Commission in Nov 2025

2.5.43 Performance of a generic SEC

- The acceptance criteria for the performance characteristics of a SEC method given in a table

Intended purpose		
Class of products	Monoclonal antibodies (excluding conjugates, fusion proteins, Fab fragments)	
Quality attributes	% HMWS and % monomer	
Type of testing	Detection and quantitation of peaks corresponding to HMWS and the monomer in monoclonal antibodies in accordance with performance characteristics and acceptance criteria below	
Result expression	% monomer and % HMWS expressed as % sum area of the peaks eluting before the peak due to the monomer, with a reporting threshold of 0.10 % (Procedure A) and 0.25 % (Procedure B)	
Performance characteristics	**Acceptance criteria**	
Specificity	Differentiate peaks due to HMWS and LMWS from the peak due to the monomer (CRS)	
	No visible interference from the blank	No peak eluting that could interfere with the analyte No peak > 0.10 % (Procedure A) no peak > 0.25 % (Procedure B) of total peak area of CRS
	Sufficient resolution between the monomer and preceding peaks obtained with CRS	

2.5.43 Performance of a generic SEC

Performance characteristics	Acceptance criteria	
Precision	Using dilutions of CRS Procedure A: 100% 10 mg/ml Procedure B: 100% 4 mg/ml	
	Repeatability:	
	– Monomer response: 3 levels in the range of 80-120 %	Max. RSD (n = 3) per level: 5 per cent
	– HMWS response: 3 levels in the range of 0.5-7.5 %	Max. RSD (n = 3) per level: 10 per cent
	Intermediate precision:	
	– Monomer response: 3 levels, range 80-120%	Max. RSD (n = 3) per level: 10 per cent
	– HMWS response: 3 levels, range of 0.5-7.5 %	Max. RSD (n = 3) per level: 15 per cent
Accuracy	Using dilutions of CRS (as above)	
	– Monomer response: 3 levels in the range of 80-120 per cent	Monomer response accuracy: 95-105 per cent
	– HMWS response: 3 levels in the range of 0.5-7.5 per cent	HMWS response accuracy: 90-110 per cent
Reportable range	Linearity of response using dilutions of CRS	
	– Linearity of monomer response: 5 levels in the range of 80-120 %	$R2 > 0.99$
	– Linearity of HMWS response: 5 levels in the range of 0.5-7.5 %	$R2 > 0.99$
	Validation of lower range limits: QL through signal-to-noise determination	Corresponds to a S/N ratio of 10

2.5.43 Size exclusion chromatography for recombinant therapeutic monoclonal antibodies

- The chapter is not mandatory *per se*
- The procedures are described in detail
  - The system performance criteria differ form HPLC to UHPLC
  - The system suitability criteria are the same for both procedures
  - Data analysis- and Results- chapters are the same
- When used as is or when they will be referred in a monograph, *5.26 Implementation of pharmacopoeial procedures* is to be followed
- If used as a starting point for development or modified, validation must be performed
- Performance characteristics provided for a generic SEC procedure
- When referred to in a monograph, the methods become mandatory

Horizontal methodological chapters – building common understanding between regulators and industry

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