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Ph. Eur. Reference Standards for Recombinant Biotherapeutics – Peptide mapping and Glycan analysis

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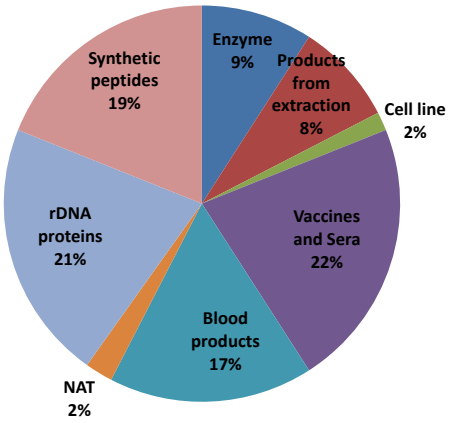
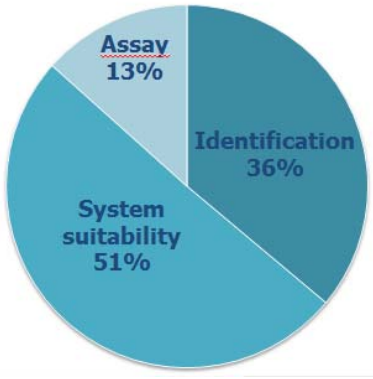
Ph. Eur. RS portfolio for biologicals

- About **140** Reference Standards for Biologicals (CRS and BRP): **4**% of Ph. Eur. RS portfolio
- **21**% monographs on rDNA biotherapeutics
- **2** categories of rDNA active substances can be identified:
  - chemically defined (small molecules). Ex: insulins
  - structurally heterogeneous (large complex, glycosylated molecules). Ex: mAb



Types of CRS for rDNA proteins

- **System suitability**
  - to verify that a measurement system is operated within the boundaries of its validation scope
    Ex: glycan analysis
- **Qualitative purpose**
  - to test compliance of essential quality attributes, i.e. identification
    Ex: peptide mapping
- **Quantitative use**
  - quantitative determination of the substance subject of the monograph
  - assigned content



Ph. Eur. reference standards are to be used as stated in a text of the Ph. Eur. They are not intended to be used as reference (comparator) products in the context of applications for biosimilars

Why is glycosylation important for biotherapeutics?

1) Glycosylation impacts properties of the protein:
   - Bioactivity
   - Pharmacokinetics
   - Stability
   - Immunogenicity

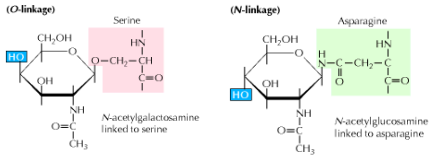
2) Glycosylation:
   - Non-template-driven enzymatic modification
     -> glycan heterogeneity

3) Heterogeneity impacts product quality:
   - Batch to batch variability
     - inconsistency of production
     - risk of out of specification

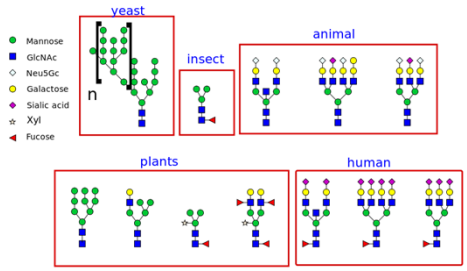
➡ Glycosylation must be: qualitatively and quantitatively controlled at all stages of the lifecycle and therefore must be controlled by the monograph

Heterogeneity of the protein glycosylation

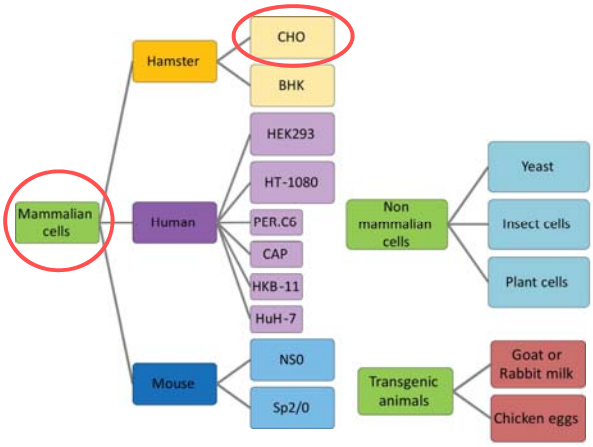
1) Two major types of glycosylation: O- and N-linked



2) Microheterogeneity in N-linked glycans



3) Expression system for rDNA proteins



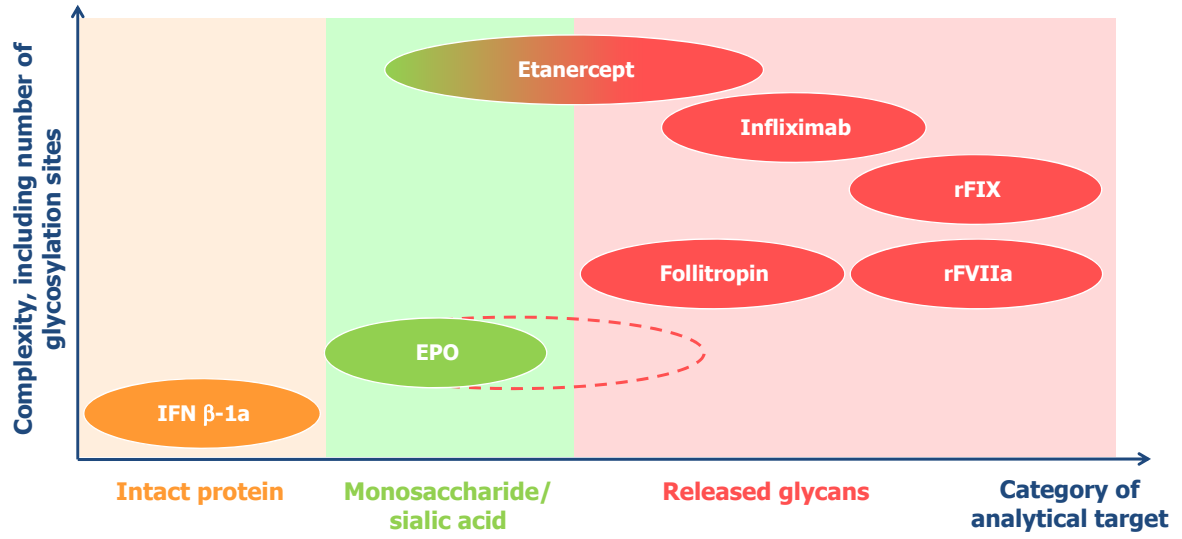
Glycan analysis procedures in the Ph. Eur.

General chapter: 2.2.59. Glycan analysis of glycoproteins

Heterogeneity in glycosylation is assessed by 4 distinct and complementary approaches:

Analytical target	Structure	Resulting information
Intact glycoprotein		overall pattern of glycosylation of the glycoprotein, limited information when the molecule is large and contains multiple glycosylation sites
Glycopeptides		site-specific glycosylation properties, degree of occupancy, oligosaccharide structures
Released glycans:		populations of glycans present on the protein (bi-, tri-, and tetra-antennary profile), degree of sialylation
labelled		
unlabelled		
Monosaccharide:		monosaccharide composition of a glycoprotein
labelled		
unlabelled		

Glycan analysis in Ph. Eur. individual monographs



Intended purpose of Ph. Eur. RS in glycan analysis

To control the performance of the method, including glycan cleavage, recovery and analysis

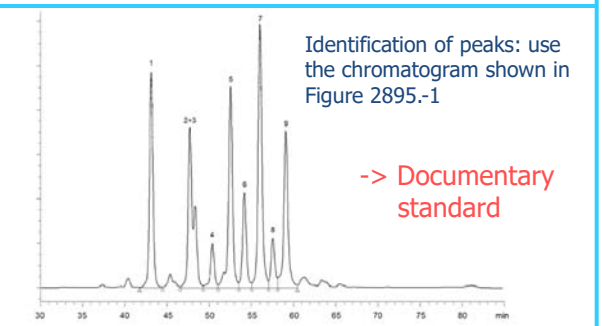
-> system suitability

"The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system" Ph. Eur. 2.2.46.

Confirmation of identity of the analytical target

Means: chromatogram included in the monograph

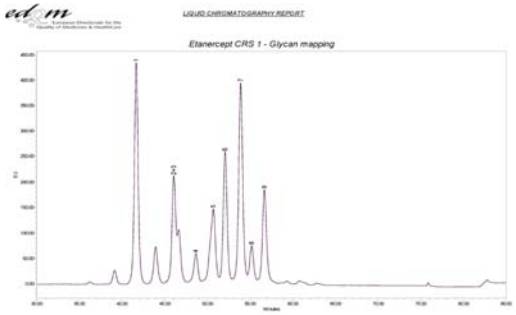
Etanercept monograph – Glycan mapping



Identification of peaks: use the chromatogram shown in Figure 2895.-1

-> Documentary standard

Etanercept CRS



-> Material standard

Confirmation of compliance with qualitative requirements

Means: CRS for system suitability and in-house reference preparation

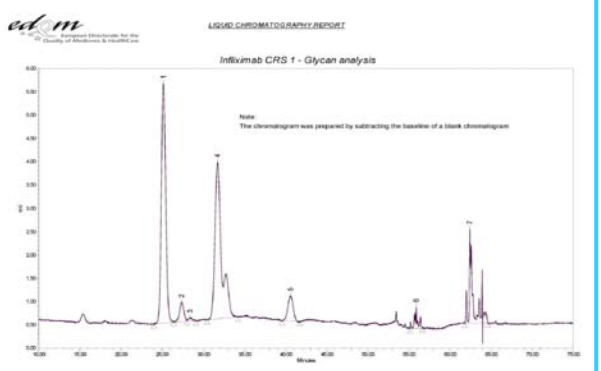
Infliximab monograph – Glycan mapping

- System suitability:
- the chromatogram **obtained** with infliximab CRS is qualitatively similar to the chromatogram **supplied** with infliximab CRS and peaks 1 to 7 are clearly visible

- Results:
- the profile of the chromatogram and the retention times of the peaks obtained with the test solution corresponds to that obtained with the chromatogram obtained with a suitable infliximab in-house reference preparation

-> Consistency of production using a production process specific reference standard

Infliximab CRS

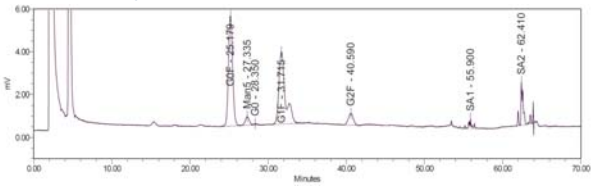


Confirmation of compliance with qualitative requirements

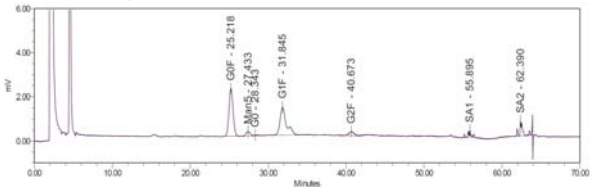
Monograph is applicable to Infliximab produced in mammalian cell expression system

Sp2/0 cell line

Infliximab, manufacturer 1

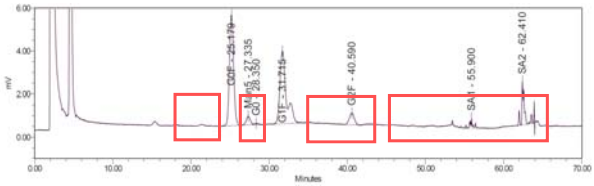


Infliximab, manufacturer 2



CHO cell line

Infliximab, manufacturer 3 *(simulation, expected differences)*



- **Infliximab CRS** is used for system suitability
- For consistency of production purpose, an in-house reference preparation is required

Peptide mapping: identification is not structure elucidation

- The elucidation of structure, which involves extensive characterisation of the substance using for ex. mass spectrometry is part of the regulatory filing, not part of testing in a monograph

- **Ph. Eur. general notices**: the tests given in the Identification section are:
  - not designed to give full confirmation of the chemical structure or composition of the product
  - intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label

CRS for peptide mapping

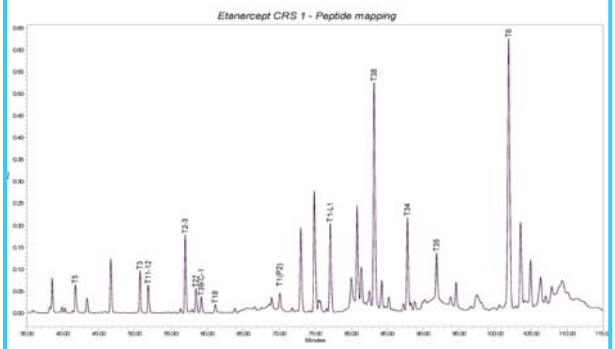
Peptide mapping:

- involves enzymatic or chemical treatment to form peptide fragments (at specific cleavage sites) that are separated (e.g. by LC) and identified
- fingerprint of a protein
- comparative procedure with CRS: by comparing the info obtained with a CRS treated similarly, the primary structure (sequence) of the protein can be confirmed and alterations can be detected

Compliance with the Ph. Eur. is a mandatory requirement (no flexibility)

Etanercept CRS for peptide mapping

Means: CRS for system suitability and peak identification

Etanercept monograph – Peptide mapping	Etanercept CRS
LC method is described in the monograph, where *Etanercept CRS* is used to prepare the reference solution The profile obtained with the test solution corresponds (in retention times, peak responses, number of peaks, overall elution pattern) to that obtained with the reference solution	

Key messages

- Usefulness of CRS for rDNA proteins
- Relevance of different CRS types:
  - to control the performance of the method: role extended
  - to assess acceptance criteria (qualitative, quantitative)
  - to allow independent testing
- Acceptance criteria in the monograph are specific (i.e. measurable attributes) and not embedded in a particular batch of candidate CRS

  Otherwise when the Ph. Eur. RS is replaced, the new batch may not be able to satisfy the same need without impacting the acceptance criteria

Key messages

- Ph. Eur. RS is just "a" material:
  - not necessarily related to the reference product
  - not necessarily related to the monograph specifications
  - is fit for the intended purpose

- Glycan analysis:
  - flexibility is built in and monograph provides means for transferability of the analytical procedure
    -> *CRS for SST*

- Peptide mapping:
  - no flexibility, mean to confirm identity of rDNA protein
    -> *CRS for SST and peak id*

Thank you for your attention

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