Application Note: Automation of Peptide Mapping Pretreatment

Introduction

Peptide mapping is a method for characterizing protein biopharmaceuticals. It relies on fragmenting the protein by a series of chemical and enzymatic treatment steps. The fragmented products are then analyzed by HPLC, coupled to UV-VIS or MS detection.

The result is a “fingerprint” that contains a wealth of information about the amino acid sequence, post-translational modifications, glycosylation profile and other distinguishing marks of the drug product. The information can be used to R&D ends as well as for QC purposes (e.g., to establish comparability between the original mAb drug and a biosimilar product).

This document describes automation of the sampling, sample clean-up and sample fragmentation processes, using the FIAlab ProSIAmpler instrument.

Principle

The FIAlab ProSIAmpler sampling device / fluidic analyzer draws a sample of culture fluid from a bioreactor, in an aseptic manner. The sample is passed through a Protein A column to capture the mAb drug product and remove the sample matrix.

The captured mAb is eluted and its UV absorbance is measured to determine the product titer. The titer value is used to scale the amount of sample used for subsequent treatment steps.

The purified sample is first denatured by the combination of heat and addition of a buffer solution. Such conditions cause the high-order structure of the protein to unravel and expose the internal chemical groups, so that further chemical manipulation and fragmentation becomes possible.

Next, a reducing agent is added to cleave disulfide bonds. This is done to prevent inter- and intra-molecular disulfide formation, which could compromise the quality of the fragmentation process.

Finally, a digestion agent is added to cleave the protein at specific sites and thus create a predictable and repeatable fragmentation mixture. The protocol uses trypsin for this purpose. Trypsin is a proteolytic enzyme that cleaves proteins at specific peptide bond sites (at arginine (Arg) or lysine (Lys) units, unless those are followed by proline (Pro)).

Finally, the digested mixture is fed into the sample loop of an HPLC instrument, and the LC is triggered to inject and analyze the treated sample.

Application benefits

Automation	→	On-line operation possible
	→	Reduced variability
Real-time operation	→	Immediate result availability

Experimental

Reagents

Denaturation: Buffer solution

Reduction: TCEP (tris(2-carboxyethyl)phosphine)

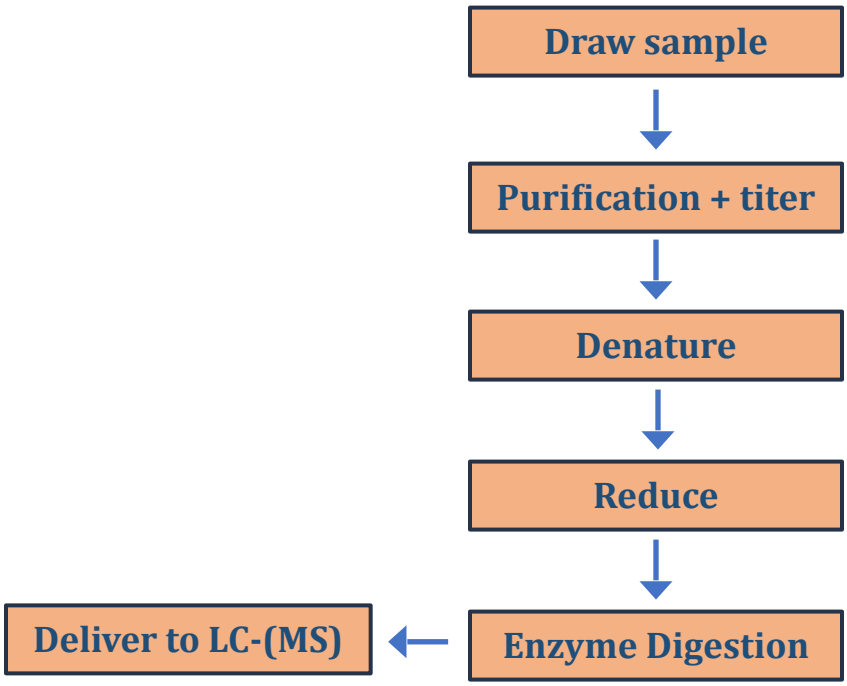
Fragmentation: Trypsin

Instrument Configuration

Analyzer: FIAlab ProSIAmpler

Titer Detection: 280 nm

Sample Processing Sequence



Conclusions

The FIAlab ProSIAmpler analyzer is capable of automated peptide mapping of a biopharmaceutical drug product. Specifically, the setup automates the following sequence of steps:

- Aseptic sampling from a bioreactor.
- Purification of the sample using a small-volume Protein A column.
- Titer determination of the purified sample. Immediate titer feedback to adjust the amount of sample processed for the peptide mapping protocol.
- Heat denaturation and reductive breakage of disulfide bonds.
- Fragmentation by proteolytic digestion with trypsin.
- Delivery of the digested sample to an LC for separation and analysis.

Besides peptide mapping, the protocol is directly applicable to Multi-Attribute Methods (MAM) where sample processing is similar, but detection is performed by a high-resolution accurate mass (HRAM) mass spectrometer.

In addition, the ProSIAmpler can be configured to perform peptide mapping / MAM methods that differ from the protocol presented here. As an example, traditional protocols often use an alkylation step following disulfide reduction, in order to prevent the disulfide bonds from re-forming after the reducing agent is removed. The ProSIAmpler can be set up to include alkylation (or other similar steps) in the sample workflow.