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LC-MS screening assays based on GSH and GSSG trapping to detect protein-reactive extractables

The common approach in extractables and leachable (E&L)-studies is to identify extractables, present above a given threshold, and to perform a toxicity assessment of these compounds with regard to potential adverse effects on humans after administration.
For biopharmaceuticals, there is another aspect of adverse effects of E&L's, the interaction with peptides/proteins. Covalent binding with reactive E&L's may affect product quality and safety, e.g. reduced efficacy or immunogenicity.
„Most, if not all, nonenzymatic covalent reactions between proteins and organic compounds are nucleophilic substitutions in which the functional groups of amino acids side chains serve as the nuclophile and the chemical modifier is the electrophil" [1].

Glutathione (GSH) reacts with a wide variety of electrophilic extractables like α-ß unsaturated carbonyls (e.g.acrylates, BHT-quinonemethide), epoxides (e.g. bisphenol diglycidyl ether), and activated halogenated compound (e.g. bromo butyl rubber oligomers) [2].
However, there is another group of protein-reactive compounds, those which are able to attack disulfide bridges in proteins like vulcanization agents of the Vultac© family. Such compounds can be trapped with GSSG, the disulfide of GSH.

ASAS offers, in collaboration with A&M Stabtest, three screening assays, based on GSH- and GSSG-trapping.
Extracts of packaging or single use material are treated with GSH and/or GSSG, the reaction products are identified using high-resolution accurate LC-MS.
Extractables, which can be converted into reactive species (pro-electrophiles) by e.g. aging processes or γ-irradiation, are trapped with GSH after electrochemical activation [3].
If a compound was found to be reactive in one of the above-mentioned screening methods, there is a certain likelihood to react also with therapeutic proteins. The knowledge of the identity of reactive compounds is essential for the characterization of potentially modified proteins.
A&M Stabtest, a GMP-certified CRO, is specialized in analysis of biopharmaceuticals. With their expertise and long-standing experience in protein sequencing, detection of PTMs and other methods for protein characterization, A&M Stabtest is the ideal partner to perform such investigations.

Results of the GSH/GSSG screening will help sponsors to fulfill FDA demands, as described in Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products:
"Sponsors should conduct a comprehensive extractables and leachables laboratory assessment using multiple analytical techniques to assess the attributes of the container-closure system that could interact with and degrade protein therapeutic products." [4].

[1] Li K, Rogers G, Nashed-Samuel Y, et al. Creating a Holistic Extractables and Leachables, (E&L) Program for Biotechnology Products. PDA Journal of Pharmaceutical Science and Technology. 2015 Sep-Oct; 69(5):590-619. DOI: 10.5731/pdajpst.2015.01073.
[2] Heap et al, Protein-reactive extractables a screening assay which adds additional value to the traditional E/L workflow. Pharmazeutische Industrie Jan 2018; 80(1):104-114
[3] Pro-electrophiles - a previously overlooked class of extractable with protein-reactive potential, presentation, held at Smithers E&L conference Europe 2020 on. DOI: 10.13140/RG.2.2.16360.90885.
[4] https://www.fda.gov/media/85017/download