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Facile Quantitation of Free Thiols in a Recombinant Monoclonal Antibody by Reversed-Phase High Performance Liquid Chromatography with Hydrophobicity-Tailored Thiol Derivatization

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Highlights

- **Novel and facile total free thiol quantitation method for intact mAbs**
- **RP-HPLC with N-*tert*-butylmaleimide tagging of free thiols**
- **An hydrophobicity-tailored approach to free thiol derivatization reagent selection**
- **Analytical method performance and robustness suitable for R&D and QC**

Abstract

Free thiol content, and its consistency, is one of the product quality attributes of interest during technical development of manufactured recombinant monoclonal antibodies (mAbs). We describe a new, mid/high-throughput reversed-phase-high performance liquid chromatography (RP-HPLC) method coupled with derivatization of free thiols, for the determination of total free thiol content in an *E. coli*-expressed therapeutic monovalent monoclonal antibody mAb1. Initial selection of the derivatization reagent used an hydrophobicity-tailored approach. Maleimide-based thiol-reactive reagents with varying degrees of hydrophobicity were assessed to identify and select one that provided adequate chromatographic resolution and robust quantitation of free thiol-containing mAb1 forms. The method relies on covalent derivatization of free thiols in denatured mAb1 with N-*tert*-butylmaleimide (N*t*BM) label, followed by RP-HPLC separation with UV-based quantitation of native (disulfide containing) and labeled (free thiol containing) forms. The method demonstrated good specificity, precision, linearity, accuracy and robustness. Accuracy of the method, for samples with a wide range of free thiol content, was demonstrated using admixtures as well as by comparison to an orthogonal LC-MS peptide mapping method with isotope tagging of free thiols. The developed method has a facile workflow which fits well into both R&D characterization and quality control (QC) testing environments. The hydrophobicity-tailored approach to the selection of free thiol derivatization reagent is easily applied to the rapid development of free thiol quantitation methods for full-length recombinant antibodies.

Abbreviations