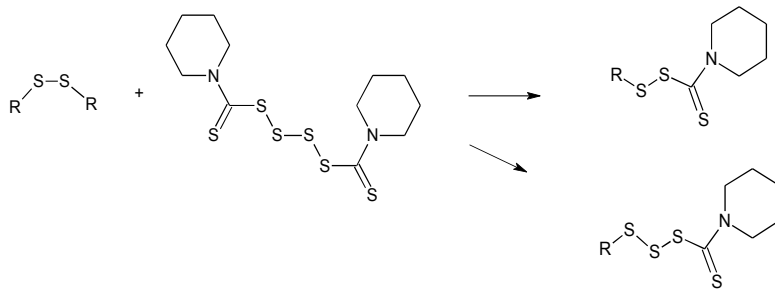


Protein-reactive extractables/leachables: Reactivity of Thiurams (vulcanization agents) towards disulfide bonds.

Summary:

Tetramethylthiuram disulfide and Dipentamethylenethiuram tetrasulfide (common rubber vulcanization agents) as well as dipropyl trisulfide (used as a model system) were able to react with GSSG to form mixed di- and tri-sulfides.

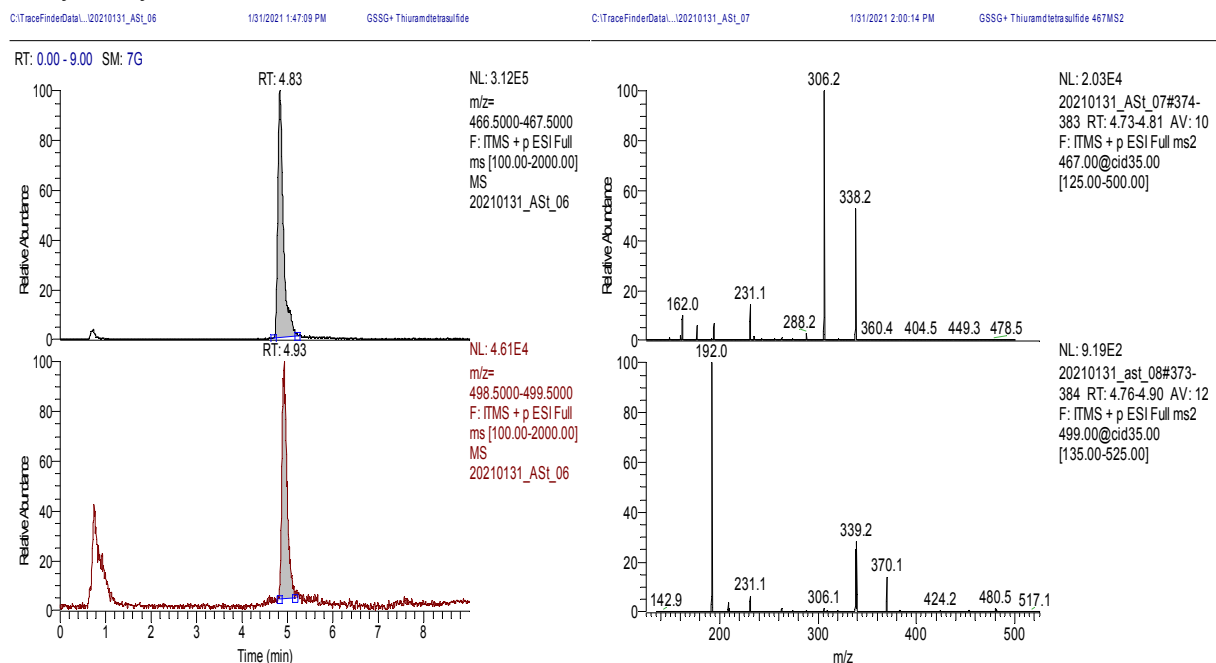


Reaction scheme of Dipentamethylenethiuram tetrasulfide

We recently described a screening assay, based on trapping of disulfide bond reactive compounds with glutathione disulfide (GSSG). Vultac7™, a vulcanization agent, was shown to react with [GSSG](#) and also with proteins like Insulin and EPO (DOI: [10.13140/RG.2.2.16360.90885](#))

Disulfide bonds are essential structure elements in peptides/proteins to maintain the 3D-structure. If a extractable/leachable is able to modify disulfide bonds in therapeutic peptides/proteins, efficacy and safety might be impacted.

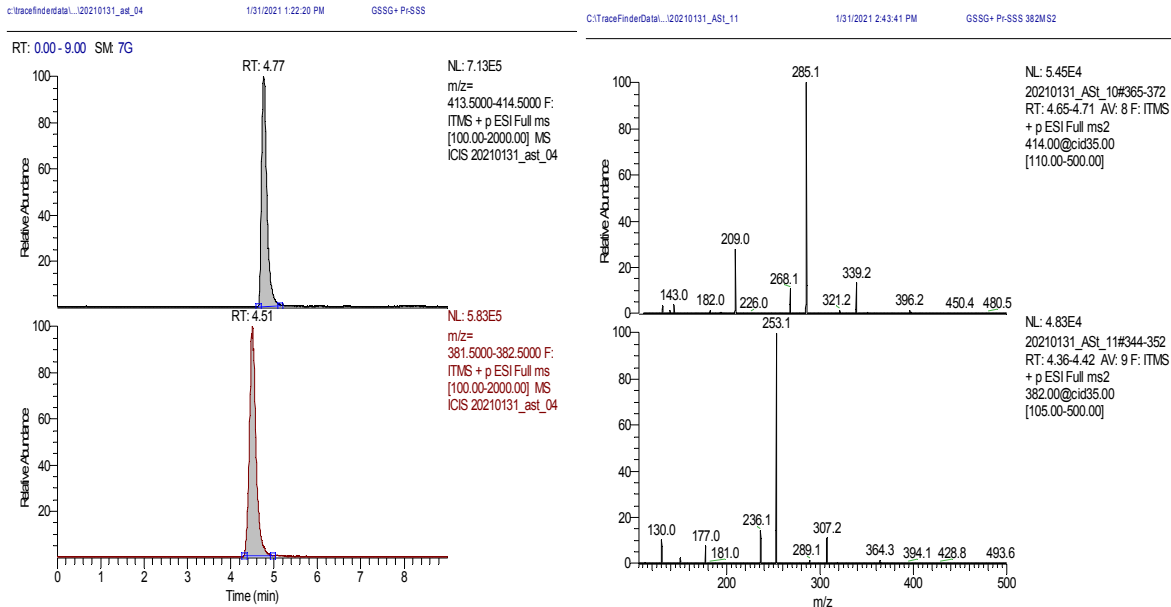
To investigate, whether other vulcanization agents can also react with disulfide bonds, two thiurams were treated with GSSG. Both thiurams formed mixed di- and trisulfides. The reaction products were analysed by LC-MS and LC-MS/MS.



Dipentamethylenethiuram tetrasulfide + GSSG
LC-MS m/z 467 (disulfide, top) and m/z 499 (trisulfide, bottom)

MS/MS of m/z 467 (top) and m/z 499 (bottom)

Dipropyl trisulfide is not extractable, but was chosen as a model system for trisulfides. Again, the mixed di- and trisulfides were found after reaction with GSSG.



Dipropyl trisulfide + GSSG
LC-MS m/z 414 (trisulfide, top) and m/z 382 (disulfide, bottom)

MS/MS of m/z 414 (top) and m/z 382 (bottom)

Conclusion:

Disulfide bonds are important structure elements of many peptides and proteins. Those bonds can be attacked by rubber vulcanization agents like Vultac™ (Alkylphenol polysulfide) or thiurams. In general, tri- and polysulfides and related compounds seem to be reactive.

GSSG was successfully applied to screen for disulfide bond reactive compounds. The GSSG screening assay is a useful additional tool for the risk assessment of extractable/leachables in biopharmaceuticals.