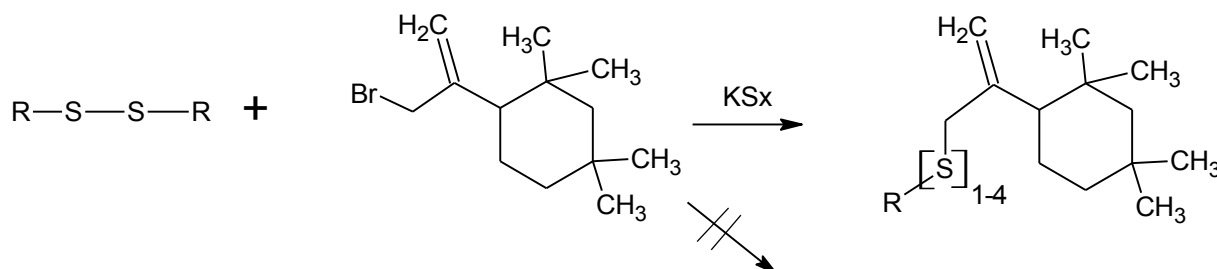
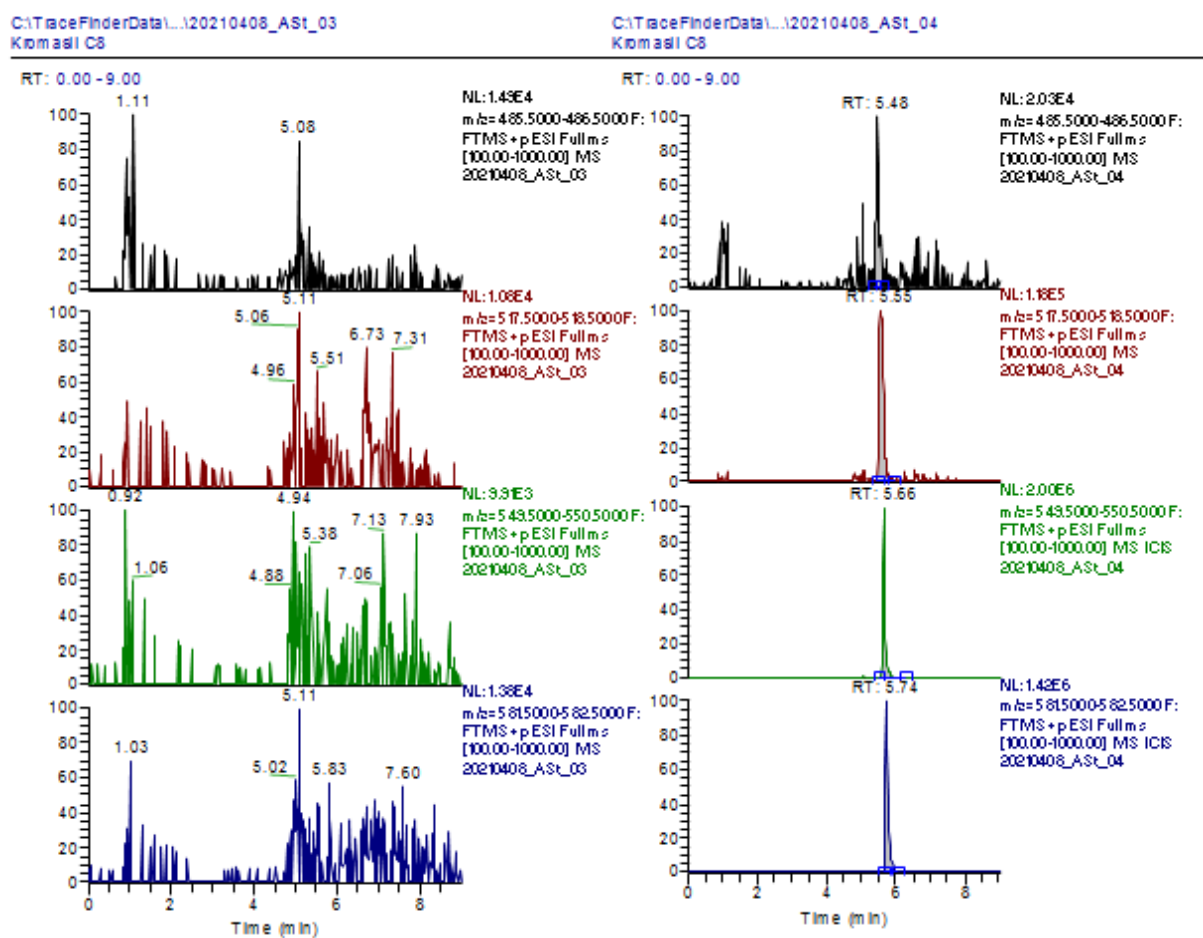


Impact of inorganic polysulfides on protein adduct formation by extractables/leachables



We recently described a screening assay, based on trapping of disulfide bond reactive compounds with glutathione disulfide (GSSG). Vultac7™ as well as Thiurams (common vulcanization agents), were shown to react with [GSSG](#) and (in the case of Vultac7™) also with proteins like Insulin and EPO (DOI: [10.13140/RG.2.2.16360.90885](#)). To investigate, whether inorganic polysulfides can also react with disulfide bonds, we treated GSSG with the rubber oligomer C13H23Br in the presence and absence of potassium polysulfides (KS_x). No reaction occurred in the absence of KS_x, but multiple products, consisting of GS-S₁₋₄-C13, were found, if KS_x was present.



No KS_x + KS_x
m/z 486 (GS-C13), 518 (GSS-C13), 550 (GSSS-C13), and 582 (GSSS-C13), from top to bottom

Inorganic polysulfides can occur in sulfur-cured rubber [Roethemeier]. In contrast to elemental sulfur (S₈), which is sometimes analyzed in the course of E/L studies [Zhang 2004], there are no publications regarding the analysis of polysulfides as extractables. We could demonstrate, that K₂S₂O₈ attacks disulfide bonds and the intermediates (probably sulfides and persulfides) can further react with electrophilic E/L's like halogenated rubber oligomers. In a bromo butyl rubber (pharmaceutical grade) extract, treated with GSSG, GSS-C13 and GSSS-C13 were identified as reaction products.

Conclusion:

- Disulfide bonds can be modified by extractables/leachables. So far, the „N- and C-terminus as well as functional side chains of arginine, lysine, histidine, cysteine, tyrosine, glutamic acid, and aspartic acid“ of peptides and proteins were regarded as E/L targets [Li 2015].
- Mixtures of extractables can result in reaction products, different from those of a single compound. This finding may impact risk assessment strategies.
- Inorganic polysulfides can impact E/L profiles, but are not monitored in E/L's studies yet.
- By application of the GSSG screening assay, the interaction of polysulfides with halogenated rubber oligomers could be revealed, showing the usefulness of such screening methods.