4. Conclusion

The new methods for synthesis and isolation of pure regioisomers of the SO-Cys, SO-Lys and SO-His adducts were developed. S-(2-Hydroxy-1-phenylethyl)cysteine and S-(2-hydroxy-2-phenylethyl)cysteine were prepared by direct alkylation of cysteine with pure enantiomers of SO. The SO adducts of lysine and histidine were prepared by alkylation of Nα-Boc protected amino acids with (*R*)-SO or (*S*)-SO enantiomers. After the Nα-Boc group deprotection, Nε-(2-hydroxy-1-phenylethyl)lysine, Nε-(2-hydroxy-2-phenylethyl)lysine, Nπ-(2-hydroxy-1-phenylethyl)histidine, Nπ-(2-hydroxy-2-phenylethyl)histidine, Nτ-(2-hydroxy-1-phenylethyl)histidine and Nτ-(2-hydroxy-2-phenylethyl)histidine were obtained.

Individual regioisomers of the SO adducts were separated and isolated by semi-preparative HPLC and their structure was characterized by NMR methods. Deuterated analogues of the SO-Cys and SO-His adducts were synthesized as well, and used as internal standards in quantitative studies.

The enzymatic hydrolysates of samples of SO-modified human globin were directly analyzed by LC/MS or dried, derivatized with TBDMS reagent and analyzed by GC/MS. All regioisomers of the SO adducts were detected in the samples. The

SO adducts in the hydrolysates were determined by GC/MS method using deuterated analogues of the SO adducts as internal standards. The most abundant regioisomers found by GC/MS in the samples were S-(2-hydroxy-1-phenylethyl)cysteine, Nε-(2-hydroxy-1-phenylethyl)lysine and Nτ-(2-hydroxy-2-phenylethyl)histidine. These adducts can be used as suitable biomarkers for biological monitoring of exposure to styrene and SO. Moreover, HPLC method was able to separate both pairs of SO-Cys diastereomers and one pair of SO-His diastereomers.

SPE method suitable for extraction of the SO adducts from human globin hydrolysate was optimized. This method was able to extract relatively low polar SO adducts from other more polar amino acids. The SO adducts were concentrated in the samples by ca. two orders of magnitude. The SPE method was used together with GC-FID to determine the SO adducts in the samples of SO-modified human globins (SO-Gb (*R*)-SO 0,625:1, and SO-Gb (*S*)-SO 0.625:1). Levels of the SO adducts in these samples were in the interval of ca. 0,4-2,0 μmol/g globin.

Sensitivity of the developed analytical protocol might be further improved by using mass spectrometric detectors instead of less sensitive flame-ionization detector. The developed method will be in future applied for determination of the SO adducts in animals treated with SO *in vivo*. The method will be also adapted

for biological monitoring of people profesionally exposed to styrene and SO. In this way, it will be possible to study the correlation between the level of SO adducts in globin and the level of styrene or SO in at workplace´s air. It is supposed that the SO adduct level in such samples of globin will be ca. 10-1000 pmol/g globin.