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JMS Letters

Dear Sir,

On the use of trimethylchlorosilane in methanol for methylation of thyroxine prior to perfluoroacylation and isotope dilution–gas chromatography/mass spectrometry

For gas chromatographic analysis, thyroxine is most commonly derivatized in a two-step reaction to the *N,O*-bis (perfluoroacyl) methyl ester.[1–4] Usually, the methyl ester is formed from reaction with methanolic HCl, which is generated from introducing gaseous HCl in dry MeOH or from hydrolysis of acetylchloride dropped into MeOH. However, in our hands, this methylation reagent posed problems, in particular because we applied it for the quantitative analysis of thyroxine in serum by isotope dilution–gas chromatography/mass spectrometry (ID–GC/MS). We obtained irreproducible isotope ratios, presumably owing to the uncontrollable concentration of HCl in the reagent (e.g. dependent on the moisture content of the MeOH and on the conditions of the *in situ* hydrolysis of acetyl chloride) and/or the formation of byproducts. Sometimes, the reagent even caused cleavage of the phenyl ether bond. This was evidenced by the presence of unlabeled thyroxine after derivatization of pure [$^{13}C_6$]thyroxine in the presence of diiodotyrosine used as carrier (during derivatization the $^{13}C_6$-labeled tyrosine moiety was thus exchanged for unlabeled diiodotyrosine). Because of these problems, we developed a methylation procedure with diazomethane followed by perfluoroacylation.[5] Although the alternative methylation procedure allowed us to quantify thyroxine in serum reliably with ID–GC/MS, it was not fully satisfactory. The reasons for this were that methylation with diazomethane resulted in a different reaction product, i.e. the *O*-methyl-*N*-perfluoroacyl methyl ester derivative and lower GC/MS sensitivity (signal-to-noise ratio) and also diazomethane is very hazardous and toxic. Because of these problems, we continued to search for another methylation procedure.

Here we report the methylation of thyroxine with a reagent consisting of trimethylchlorosilane (TMCS) in methanol (Macherey–Nagel, Düren, Germany). Although this reagent is known as a methylation agent, to the best of our knowledge it has never been described for the derivatization of thyroxine. The optimized methylation conditions consist of reaction in 100 µl of methanol and 30 µl of TMCS at 70 °C for 1 h. The advantages of the proposed methylation procedure are multiple: it is based on the same methylation reagent (methanolic HCl) as used before,[1–4] and thus also results in the *N,O*-bis(perfluoroacyl) methyl ester; however, the *in situ* production of HCl from TMCS in MeOH is easy and user-friendly to perform and apparently happens in a more controlled way, since reproducible isotope ratios are obtained; compared with methylation using diazomethane, it results in better GC/MS sensitivity.

We have used TMCS in methanol in our ID–GC/MS method[5] for thyroxine quantification in 31 human sera with concentrations ranging between 40.6 and 373 nmol l^{-1}. Using the method under the conditions of a reference method,[6] we were able to satisfy the proposed analytical specifications, i.e. a maximum total analytical error of 3%, a maximum total imprecision ($n = 6$) of 2% and a maximum bias of 0.9%. From these results, we recommend the use of TMCS in MeOH for the methylation of thyroxine prior to perfluoroacylation and quantitative ID–GC/MS analysis.

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Yours,

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