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Discussion

Reply to "Suitability of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide as derivatization reagent for the determination of the estrogens estrone and 17α-ethinylestradiol by gas chromatography-mass spectrometry"

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We thank Zuo and Zhang [1] for their thoughtful discussion of our paper [2]. Our original communication highlighted that there was a problem with the use of *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) or *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) as silylation reagents under reaction conditions commonly used for simultaneous determination of estrone (E1) and 17α-ethinylestradiol (EE2) by GC–MS. The resulting trimethylsilyl (TMS) and *t*-butyldimethylsilyl (TBS) derivatives of EE2 were partially converted to their respective E1 derivatives. Despite the fact that BSTFA and MTBSTFA have been used extensively to derivatise EE2 and E1 in various solvents with and without catalysts, breakdown of the derivatives has not been reported in any previous work. In reply to Zuo and Zhang's discussion of our paper we would like to make the following comments:

(1) It was not the intention of our communication to present a detailed evaluation of derivatization conditions, nor to imply that silylation of EE2 using BSTFA or MTBSTFA is impossible—we simply wanted to point out that some of these methods need further evaluation.
(2) We did not set out to give an exhaustive review of the literature, and therefore the criticism that we did not refer to Liu et al. [3], Thorpe et al. [4] or Spengler et al. [5] is a little unfair because either those papers were not published at the time of our original submission, or they do not refer to the specific derivatisation procedures in question. While we accept that Zuo and Zhang may have been using BSTFA + 1% TMCS for a long time, we cannot find detailed descriptions of their procedures in the current literature (the only references pertaining to derivatisation of EE2 we can find are conference abstracts).
(3) It is clear from Zuo and Zhang's discussion paper that pyridine solvent improves the derivatisation procedure. They have not, however, identified the small peak at about 16.5 min in the TIC of TMS-EE2, which, we think, represents TMS-E1. While this peak is not significant in this case, our original communication showed that conversion can be variable. We recently submitted a manuscript (not yet published) to this journal that reports on the systematic optimisation of derivatisation conditions using common silylation reagents and solvents. We can now confirm that silylation of EE2 using BSTFA + 1% TMCS in pyridine solvent (1:1 volume ratio reagent to solvent) gives good results, but only if appropriate reaction conditions are selected. We found, for example, that TMS-EE2 was partially converted to TMS-E1 (Fig. 1) when the reaction was carried out at 75 °C for 30 min. Peak 1 in the TIC (Fig. 1a) corresponds to TMS-E1 (mass spectrum Fig. 1b), while peak 2 represents 3, 17-di-TMS-EE2 (mass spectrum Fig. 1c).

Again we thank Zuo and Zhang for their useful comments and feel that the discussion of this matter will improve analysis methods for these hormones by GC–MS. We wish to emphasise the importance of optimising reaction conditions for silylation of estrogens—our recent work shows that conditions such as temperature, time, and choice of derivatisation solvents or reagents must be carefully selected to avoid conversion problems.

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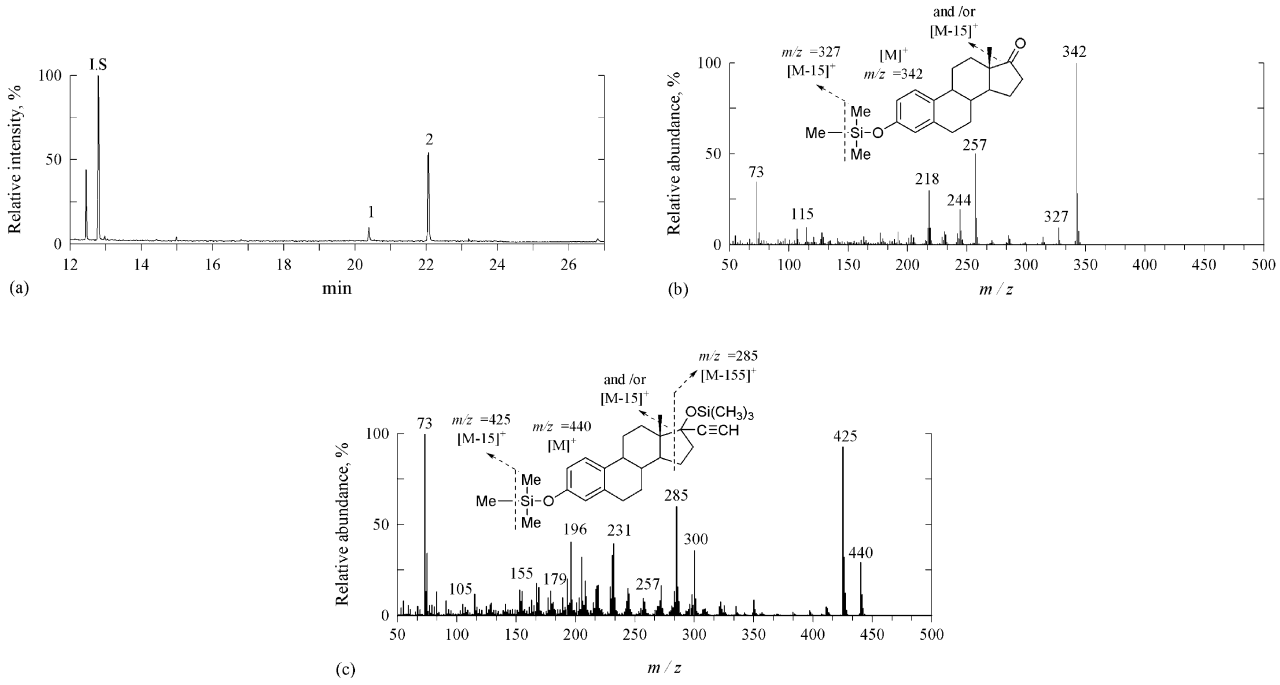

Fig. 1. GC–MS TIC of: trimethylsilyl (TMS) ethinylestradiol (EE2) (a); mass spectra of TMS-E1 (b); and di-TMS-EE2 (c). The proposed fragmentation patterns are shown above the respective mass spectrum. The internal standard (I.S.) was anthracene. Derivatization conditions: 75 °C, 30 min using BSTFA + 1% TMCS.

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