

Discussion

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

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In a recent contribution to this journal [1], Shareef et al. have investigated the use of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) for derivatizing estrone (E1) and 17 α -ethinylestradiol (EE2) and found that the resulting trimethylsilyl (TMS) and *t*-butyldimethylsilyl (TBS) derivatives of EE2 were partially converted to their respective E1 derivatives. Therefore, they concluded that these reagents may not be suitable for simultaneous determination of estrogens in environmental samples and raised questions about the reliability of earlier results reported. Their conclusion has been cited in a recent review paper [2]. Although the authors have revealed a significant problem in the derivatization and GC–MS determination of EE2 in some previous studies, we have found that BSTFA gives good results when the derivatization is carried out in a pyridine solvent.

The BSTFA has been a widely used silylation reagent for the formation of trimethylsilyl derivatives in the GC determination of steroids since it was introduced by Chambaz and Horning in 1967 [3]. Catalysts such as trimethylchlorosilane (TMCS) and pyridine are usually involved in the derivatization with BSTFA [4]. Some previous studies on the GC–MS determination of estrogenic steroids in environmental samples were carried out following the analyte silylation in pyridine [5–10]. These works have not been refereed in Shareef et al.'s communication [1]. Pyridine as a catalyst and solvent not only removes the hydrogen ion produced from the products, but also activates the hydroxyl

groups. During the past few years, we have been using BSTFA as a derivatization reagent to convert EE2 and other estrogenic steroids to trimethylsilyl derivatives with pyridine as a solvent for their GC–MS analysis and never observed the conversion of the TMS derivative of EE2 to the E1 derivative [8]. The following describes the general analytical procedures used in our study.

One hundred microliters of 0.1 mg/mL of standard solution was pipetted into a 2-mL minivial and dried by a stream of nitrogen. The standard or prepared dry sample was then derivatized by the addition of 50.0 μ L of pyridine and 50.0 μ L of the derivatization reagent BSTFA + 1% TMCS (Supelco Inc., Supelco Park, PA), and heated at 80 °C for 1 h. The derivatized samples were separated on a 30 m, 0.32 mm i.d., 0.25- μ m film DB-5 fused silica capillary column (J&W Scientific, Folsom, CA) using a Hewlett-Packard (HP) Model GC 5890 Series II gas chromatograph coupled with an HP 5971 Series mass selective detector. The column temperature was initially held at 80 °C for 4 min, then programmed to 200 °C at a rate of 20 °C/min, from 200 to 300 °C at a rate of 8 °C/min, with a final hold time of 2.5 min. Helium was used as the carrier gas and the column head pressure maintained at 8.0 Psi. Injector temperature was maintained at 280 °C, and the injection volume was 1.0 μ L in the splitless mode. The electron multiplier voltage for MS was 1988 V, and the transfer line temperature was held at 280 °C. Mass spectra were scanned from *m/z* 50 to 650 at a rate of 1.5 scans/s. Fig. 1A shows a typical GC–MS total ion chromatogram (TIC) of trimethylsilyl 17 α -ethinylestradiol and the internal standards. The mass spectrum from the di-trimethylsilyl EE2 peak is presented in Fig. 1B. It indicates that pyridine as a derivatization solvent prevents the conversion of the TMS derivative of EE2 to the E1 derivative. However, when the volume ratio of pyridine

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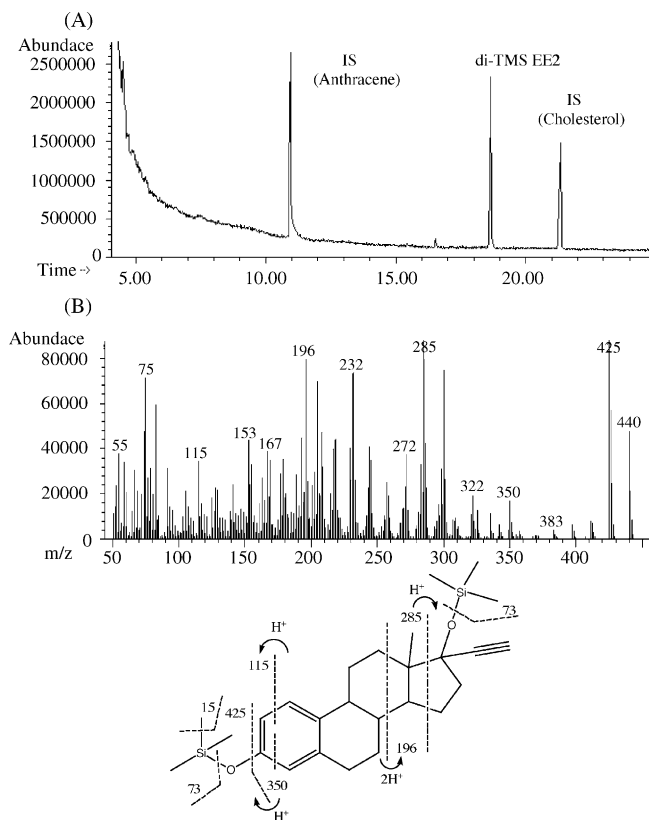


Fig. 1. (A) GC–MS TIC chromatogram of trimethylsilyl 17 α -ethinylestradiol and the internal standards anthracene and cholesterol; (B) mass spectrum from the di-trimethylsilyl EE2 peak in (A).

to derivatization reagent BSTFA + 1% TMCS was less than 1:4, partial conversion of the EE2 derivative to the E1 derivative was observed. In conclusion, the BSTFA + TMCS in the presence of sufficient amount of pyridine is a suitable derivatization reagent for the simultaneous determination of EE2, E1 and other estrogenic steroids in environmental samples using GC or GC–MS.

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