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Note

Gas chromatographic and gas chromatographic–mass spectrometric analysis of cephalexin

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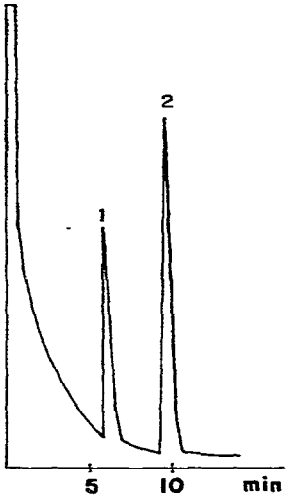
Cephalexin, 7-(D-2-amino-2-phenylacetamido)3-methyl-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid, is widely used as a broad-spectrum cephalosporin antibiotic which is orally administered. Several methods have been reported for the assay of this drug[1], including microbiological assay, chemical assay and chromatography. Microbiological assay is a tedious procedure and sometimes coexisting bioactive substances interfere. Chemical assay, often incorporated with spectrophotometry, requires pre-treatment including solvent extraction and gives a relatively poor sensitivity. The recent development of high-performance liquid chromatography has enabled cephalexin in biological fluids to be determined with high sensitivity and specificity with simple pre-treatment[2,3]. Gas chromatography (GC) has also been reported to be applicable to the indirect determination of cephaloridine[4] and the direct characterization of some cephalosporins in combination with pyrolysis[5].

In our studies on the chromatographic analysis of antibiotics, we have examined the GC of cephalexin. This paper describes the trimethylsilyl (TMS) derivatization, GC conditions and the GC–mass spectrometric (MS) identification of the TMS derivative of cephalexin.

Several combinations of TMS reagents were examined in order to convert cephalexin into the TMS derivative, which has sufficient volatility for GC. A mixture of acetonitrile, N,O-bis(trimethylsilyl)acetamide and trimethylchlorosilane (2:5:5) was found to give the best yield of the TMS derivative at room temperature, which gave a clear, single peak on the chromatogram, as shown in Fig. 1, 5α-cholestane being used as an internal standard to estimate the dependence of the yield on the reaction conditions.

Fig. 2 shows the time course of the relative yield using the same reagent mixture, indicating that the formation of the TMS derivative reached a maximum about 20 min after the initiation of the reaction, followed by gradual decrease down to 50% of the maximum at 120 min. This decrease can be related to the fact that two unidentified minor peaks appeared on the chromatogram after a long reaction time. These peaks may be due to mono- and di-TMS-substituted cephalexins. This instability of the TMS derivative suggested that the reaction should be terminated 20 min after initiation, by cooling the reaction mixture in ice–water for 5 min. The calibration plots thus obtained showed good linearity (correlation coefficient 0.999) and the detection limit was 0.1 μg per injection.



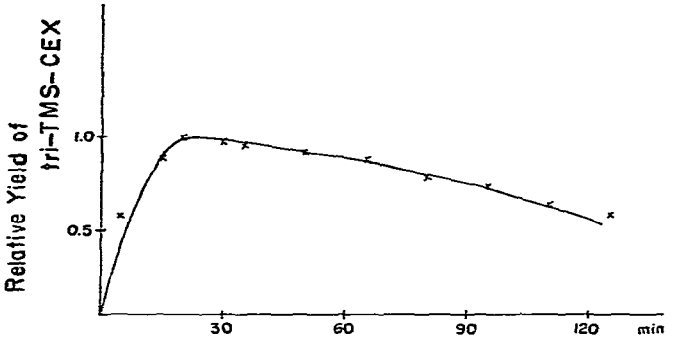


Fig. 1. Gas chromatogram of TMS derivative of cephalexin. Peaks: 1 = 5α-cholestane; 2 = TMS derivative of cephalexin. Shimadzu GC-5A chromatograph; column, 1 m × 3 mm I.D.; glass tubing packed with 1.5% OV-101 coated on Chromosorb W (60–80 mesh, AW, DMCS-treated); column temperature, 175°; injection port temperature, 280°; carrier gas (helium) flow-rate, 125 ml/min; detector, flame ionization.

Fig. 2. Relationship between reaction time and relative yield of TMS derivative of cephalexin.

The TMS derivative of cephalexin was identified by measuring the mass spectrum of the GC peak (retention time 10 min) in Fig. 1. The mass spectrum (Fig. 3) shows the formation of a molecular ion and some characteristic fragment ions, the assignments of which are summarized in Fig. 3. The results indicate that three TMS groups had been substituted on to –COOH, –NH– and $–NH_2$ groups in the cephalexin molecule.

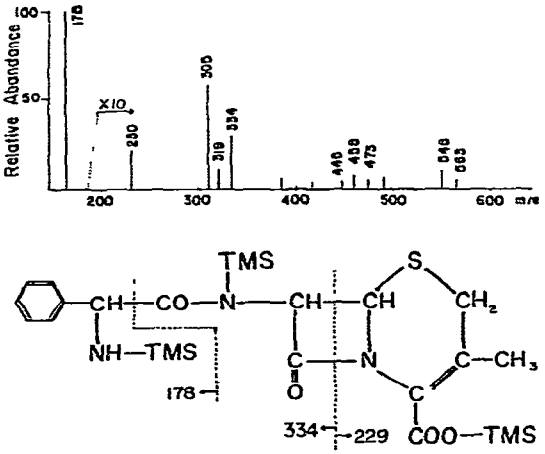


Fig. 3. Mass spectrum of TMS derivative of cephalexin. *m/e*: 563 (M^+), 548 ($M^+ - CH_3$), 473 ($M^+ - TMS - OH$), 458 ($M^+ - TMS - OH - CH_3$), 446 ($M^+ - TMS - CO_2$), 314 ($334 - CH_3$), 305 ($334 - CO$).

These results should contribute to the further development of a sensitive assay method for cephalexin in aqueous solutions and biological fluids.

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