Journal of Chromatography, 89 (1974) 80–83

CHROM. 7096

Note

Gas chromatographic determination of ethambutol

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(Received September 13th, 1973)

Ethambutol is an oral chemotherapeutic drug effective against the genus *Mycobacterium*, specifically, *Mycobacterium tuberculosis*. Several gas chromatographic methods for separating ethambutol and other antitubercular drugs have been previously reported[1,2]. However, these methods are not entirely satisfactory because a complicated derivatization process must be utilized along with a programmed temperature system. The method developed in this laboratory makes use of a single-step derivatization, isothermal temperature, and a multipurpose column. There is no interference from other antitubercular drugs that may be used in conjunction with ethambutol.

EXPERIMENTAL

Reagents

N-Trimethylsilylimidazole (TSIM), N,O-bis(trimethylsilyl)acetamide (BSA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), and hexamethyldisilazane (HMDS) (Pierce Chemical Company, Rockford, Ill. 61105, U.S.A.) were used to prepare the trimethylsilyl derivatives. Ethambutol (Myambutol®) was supplied by Lederle Laboratories, Pearl River, N.Y. 10965, U.S.A. Pyrazinamide, cycloserine, ethionamide, pyridoxine, and isoniazid, other antitubercular drugs often used in conjunction with ethambutol, were obtained from the Veterans Administration Hospital Pharmacy, Shreveport, La. 71130, U.S.A. Stock solutions containing 1 mg/ml of each drug as well as a mixture of the compounds were prepared. These stock drug solutions were refrigerated when not in use and were stable for several weeks.

Procedure

Preliminary attempts at silylation were performed at room temperature in 1-ml reaction vials fitted with PTFE-lined caps using the undiluted TMS reagents. One-tenth milliliter of each standard solution was pipetted into a 1-ml conical reaction vial and the solvent removed by evaporation in a temperature-regulated heating block. The residue was then dissolved in 0.1 ml of the various undiluted silylating reagents. One microliter of each drug derivative was injected into the gas chromatograph at 0 min, 1, 2, 3 and 4 h, and showed that only TSIM derivatized ethambutol

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immediately and completely. The other silylating agents required mild heating at 50° to complete the derivatization of ethambutol. Since TSIM reacts only with hydroxyl groups[3,4], only ethambutol and pyridoxine were derivatized because the others did not contain reactable functional groups (Fig. 1). A 1:5 dilution of TSIM in spectrophotometric-grade chloroform also derivatized ethambutol and did not affect retention time or peak symmetry but did reduce the solvent peak. The dilution also minimizes column damage.

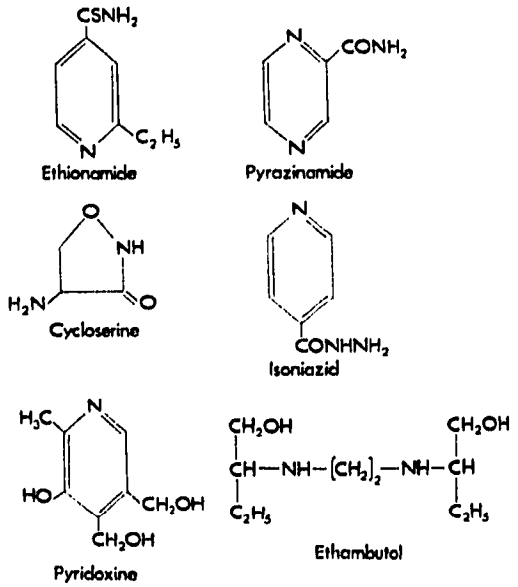


Fig. 1. Six antitubercular drugs investigated.

Gas chromatography

A Beckman GC-65 gas chromatograph (Beckman, Fullerton, Calif., U.S.A.) equipped with a flame ionization detector was used in conjunction with a Leeds and Northrup Speedomax XL 600 Series recorder (Leeds and Northrup, North Wales, Pa., U.S.A.) equipped with a disc integrator and automatic printer.

Column

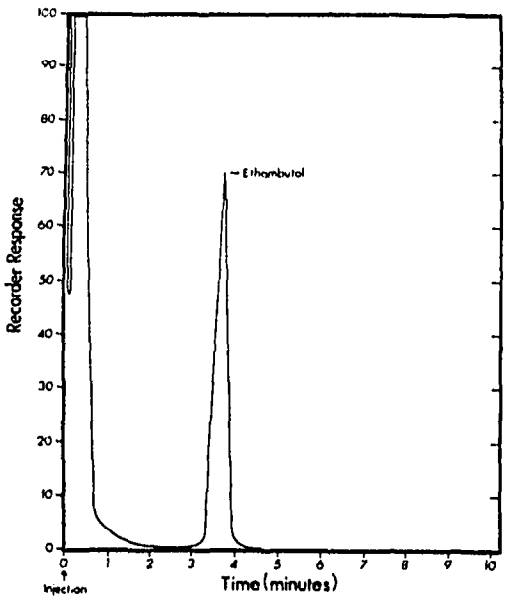
A silanized glass column, 1.5 m × 6 mm O.D. × 2 mm I.D., packed with 3% OV-17 on Chromosorb W-HP, 100–120 mesh, was used. The column was conditioned overnight at 275° with a helium flow-rate of 20 ml/min.

Temperatures

The injection port was set at 200°, the column temperature at 150°, and the detector temperature at 200°.

Flow-rates

The flow-rates utilized were: helium, 90 ml/min; hydrogen, 60 ml/min; air, 300 ml/min.



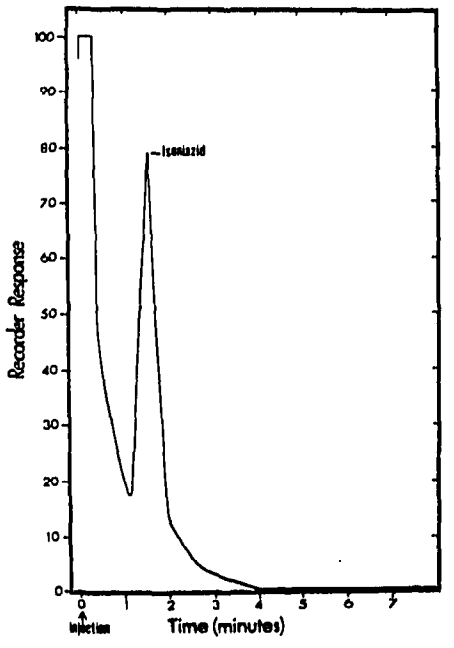
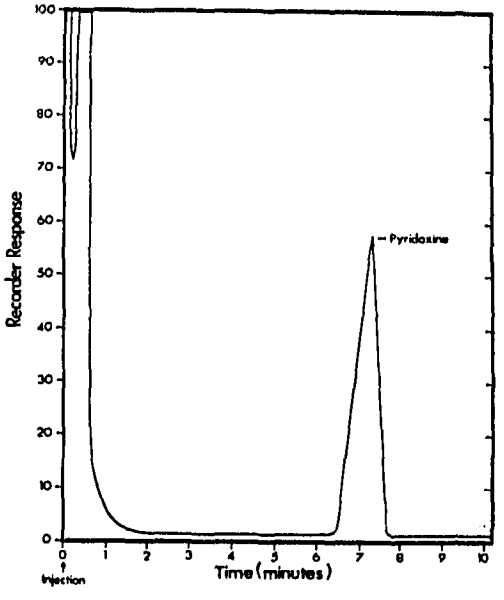


Fig. 2. Gas chromatogram of ethambutol TSIM derivative.

Fig. 3. Gas chromatogram of isoniazid TSIM derivative.



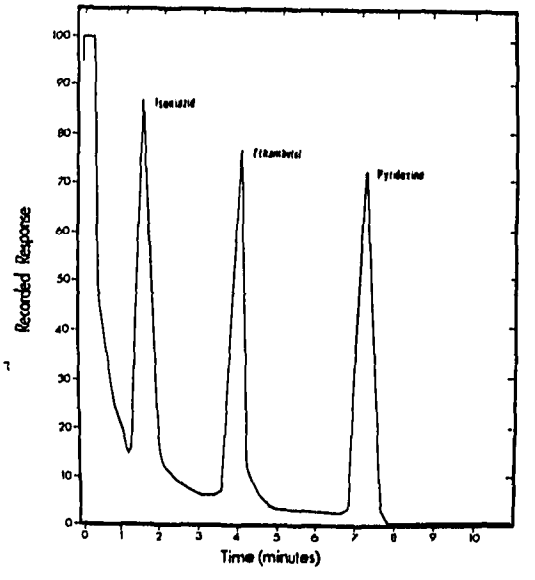


Fig. 4. Gas chromatogram of pyridoxine TSIM derivative.

Fig. 5. Gas chromatogram of a mixture containing TSIM derivatives of ethambutol, isoniazid, and pyridoxine.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, ethambutol has a retention time of 4 min (Fig. 2), isoniazid 1.8 min (Fig. 3), and pyridoxine 7.2 min (Fig. 4). The other antitubercular drugs are not eluted under the above conditions. Fig. 5 illustrates a chromatogram of a mixture of each of the drugs studied.

Several column packings were tested which could have multiple utility. A mixture of 1% OV-1 and 1% OV-17 on Chromosorb G-DMCS, a column we have found useful for chromatographing a large variety of drugs, eluted the derivatized sample but at the expense of much tailing. Thirty percent Carbowax 20M on Chromosorb W-HP, an excellent alcohol column[5], was decomposed by the silylation reagent. Neither decomposition or adsorption was encountered when 3% OV-17 on Chromosorb W-HP was used as the column packing. This column will also separate cannabinols, barbiturates, narcotics, and other drugs.

BSA, BSTFA, TMCS and HMDS were useful as derivatizing agents for ethambutol, but reacted only after mild heating or some other catalytic reaction. When TMCS was used as a catalyst in the HMDS reaction, a NH_4Cl precipitate was formed. Although the precipitate will not interfere with the chromatogram, mechanical problems will arise when small samples, such as 20 μl, are used. The sample cannot be centrifuged or withdrawn due to the excess NH_4Cl. Also, the precipitate solidifies very rapidly in the syringe. BSA, BSTFA, TMCS and HMDS are also extremely sensitive to moisture, decomposing upon contact with water. The usual precautionary measures necessary with other silylating agents with reference to water are not necessary with TSIM.

As a result of this study, a simple, rapid gas chromatographic method for determining ethambutol utilizing an isothermal temperature system and a multipurpose column has been developed.

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