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Single Derivatization Method for Routine Analysis of Bacterial Whole-Cell Fatty Acid Methyl Esters, Including Hydroxy Acids

LINDY TUPPIE MILLER
Hewlett-Packard Co., Avondale, Pennsylvania 19311

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Analysis of fatty acid methyl esters prepared from whole-cell bacteria by sodium hydroxide hydrolysis and boron trichloride-catalyzed methylation showed degradation of hydroxy acid peaks after several injections on a fused silica capillary column. A simple base wash of the extracts before injection prevented the tailing of the hydroxy acid peaks even after extended use. This eliminates the need to form trifluoroacetic anhydride derivatives of the hydroxy acids.

Fused silica capillary columns (1, 2) have been used to analyze bacterial fatty acid methyl esters (FAME) (5, 6). Tailing of hydroxy acid peaks is evident after light use, with extracts derivatized by a procedure consisting of base hydrolysis and acid methylation as described by Dees and Moss (3, 4). Although the column is no longer capable of chromatographing hydroxy acid methyl esters, FAME of other functional groups are not affected. However, the fused silica capillary column may be used extensively for bacterial FAME analysis without hydroxy acid peak tailing if the organic extracts are base washed before injection.

A bacterial FAME standard (Supelco, Bellefonte, Pa.) containing representative compounds of the functional groups common in bacteria was injected onto a fused silica capillary column (25 m by 0.2 mm) coated with SE-54 (Hewlett-Packard, Avondale, Pa.). The column was installed in a Hewlett-Packard 5880 gas chromatograph equipped with a flame ionization detector. At an initial oven temperature of 120°C, a temperature program of 8°C/min began at injection and continued to a final oven temperature of 270°C, which was held isothermal for 3 min. The injection port and detector temperatures were set at 250°C. By using hydrogen as a carrier gas, the column head pressure was set at 10 lb/in^2 to give a linear velocity of 35 cm/s. A 1-μl injection was made, using a split ratio of 100:1.

Bacterial extracts were prepared by the procedure of Dees and Moss (3, 4), which hydrolyzes whole cells with sodium hydroxide in 50% methanol. The saponified solution is acidified with hydrochloric acid and then methylated with 10% boron trichloride in methanol. Hexane-ether (4:1) was used to extract the FAME. The base wash procedure consisted of gently mixing 3 ml of 0.3 N NaOH solution with 1 ml of the organic extract, which can then be pipetted off and injected.

Figure 1 shows the chromatogram obtained when the column was first installed. Each peak is base-line resolved, with a sharp symmetrical peak shape. After 10 2-μl injections of bacterial FAME extracts derivatized by the procedure described above, another injection of the standard mix was made. Figure 2 shows the tailing of the hydroxy acid methyl ester peaks (indicated by the arrows). The tailing is assumed to be a result of residual acid and reagent being injected onto the column. The tailing and adsorption occur sooner with fused silica capillary columns than with packed columns.

A common solution to the problem of hydroxy peak tailing has been to use trifluoroacetic anhydride (TFA) to form the double derivatives of the hydroxy acids (3, 7). Although acetylation restores hydroxy peak shape, it causes a shift in retention which results in coelution of hydroxy acid double derivatives and nonhydroxy FAME. For example, C14:0 3-OH TFA coelutes with C15:0 iso, both common bacterial fatty acids. This requires two chromatographic runs, one before and one after TFA acetylation.

A less time-consuming solution to hydroxy acid peak tailing is a simple base wash of the organic extract before injection. The base wash removes residual acid and reagent which would be damaging to the column.

Figure 3 shows a chromatogram of the bacterial FAME mix run on another SE-54 fused silica capillary column (25 m by 0.2 mm) after this column had been used to analyze over 500 base-washed bacterial FAME extracts derivatized by

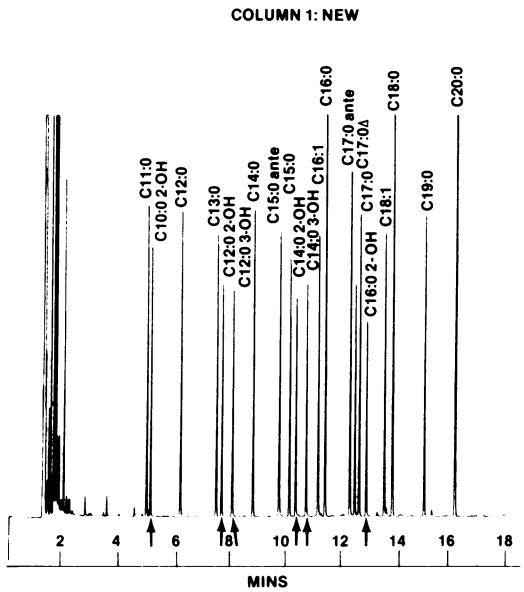

FIG. 1. Gas chromatogram of a standard bacterial fatty acid methyl ester mix separated on a new SE-54 fused silica capillary column (25 m by 0.2 mm). (See text for conditions.) All of the peaks have a symmetrical peak shape. Arrows indicate hydroxy acid FAME peaks.

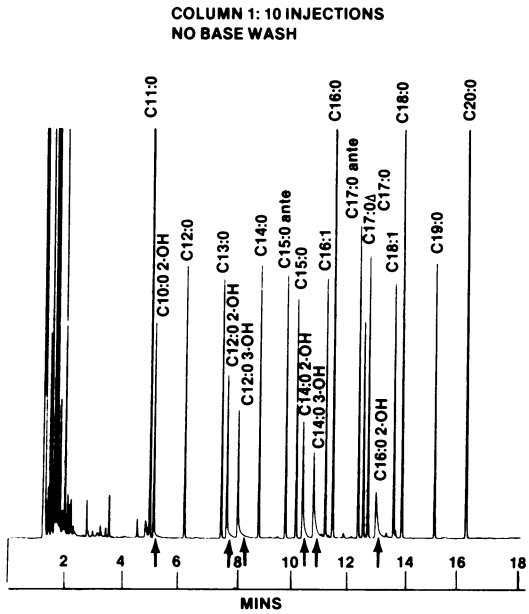

FIG. 2. Gas chromatogram of the standard mix separated on the same fused silica capillary column after light use with extracts derivatized with 10% BCl_3 in methanol as described by Dees and Moss (3) and by Moss (4). Hydroxy acid FAME peaks (indicated by arrows) are tailed.

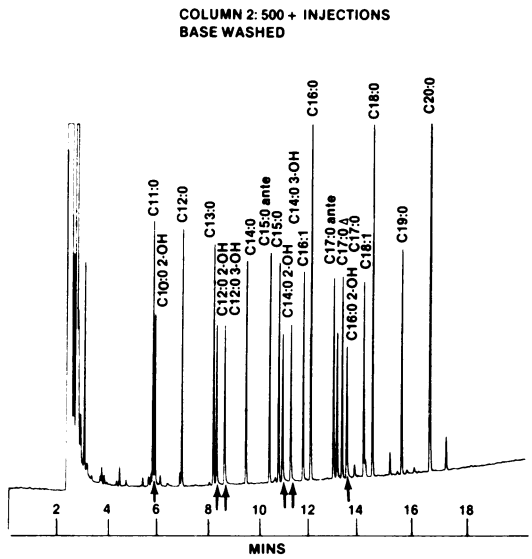

FIG. 3. Gas chromatogram of the standard bacterial FAME mix separated on another SE-54 fused silica capillary column (25 m by 0.2 mm) after extended use analyzing base-washed bacterial FAME extracts derivatized with 10% BCl_3 in methanol. Hydroxy FAME peaks show only very slight tailing.

the described procedure. Careful examination shows that the hydroxy acids are only slightly tailed.

The base wash procedure increases the lifetime of the fused silica capillary column for hydroxy acid methyl ester analysis. The technique eliminates the need for preparing double derivatives and the two chromatographic runs required by the TFA procedure. It also removes small amounts of underivatized free fatty acids resulting from incomplete methylation. Without base washing, the presence of these underivatized fatty acids in the extract can cause confusion when trying to identify the methyl ester peaks. The combination of these effects makes base washing of the organic extract a recommended step in the sample preparation of bacterial whole cell FAME extracts (6).

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