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5 Abbreviations used: DNS-$ACTH_{1-24}$, $N^{\epsilon 21}$-(dimethylaminonaphthalene-5-sulfonyl)-adrenocorticotropin-(1-24); DNS-Lys, N^{ϵ}-(dimethylaminonaphthalene-5-sulfonyl-L-lysine; $ACTH_{1-24}$, adrenocorticotropin-(1-24) tetracosapeptide (Synactin®); ANS, 8-anilino-l-naphthalene sulfonate.

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Rapid temperature programmed gas-liquid chromatography of volatile fatty acids (C_1-C_7) for the identification of anaerobic bacteria

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Summary. A gas liquid chromatography method for the separation of 10 volatile fatty acids (C_1-C_7 and isomers) has been improved by using oven temperature programmed conditions. In our conditions, the proprietary stationary phase SP 1220 introduced by Supelco Inc., gave sharp separation of volatile fatty acids in less than 8 min. This method was suitable for analyses with both thermal conductivity and flame ionization detectors.

The first application of GLC for the rapid identification of bacteria was reported in 1963[1]. Gas chromatography is now currently used for the identification of bacterial by-products in broth cultures. Whole culture and culture supernatant[2], products obtained by cation exchange column[3] or by ether extraction[4] and vapor phase of anaerobic cultures[5] can be chromatographed. Volatile fatty acids (VFA), non volatile fatty acids and alcohols produced by anaerobic bacteria may be necessary for their identification and generally the VFA analysis is the first step. Among the different GLC methods used for separation of VFA usually found in anaerobic bacterial cultures, the use of the stationary phase SP 1220 prepared by Supelco Inc. (Supelco, Inc. 1975. Chromatography/lipids. Analysis of VFA's from anaerobic fermentation. Bulletin 748E. Supelco, Inc. Bellefonte. PA) is worth mentioning. When used with a thermal conductivity detector, the SP 1220 was shown to give a better resolution and a shorter elution time of VFA (12 min for C_1-C_6 and isomers) as compared to Resoflex® column packing (12-25 min for C_1-C_6 and isomers)[6]. The SP 1220 packing was recently shown to be useful for the efficient resolution of keto VFA[7].

An ideal chromatography method should involve both an efficient and rapid resolution of the components of a sample. The present communication deals with the improvement in the rapidity of a highly efficient GLC technique for VFA analysis[6] by oven temperature programmed conditions and using the SP 1220 column packing. This method is suitable for analyses with both thermal conductivity detector (TCD) and flame ionization detector (FID).

The 4 anaerobic bacterial strains used in this study included *Clostridium histolyticum* (C 22), *Propionibacterium acnes* (Pr 20), *Bacteroides asaccharolyticus* (B 5) and *Streptococcus morbillorum* (Sc 13) from our collection. All strains were inoculated, using the glove box procedure[8], in prereduced chopped meat carbohydrate medium (CMC, Carr Scarborough Microbiologicals Inc. GA) and the cultures were incubated for 48 h at 37 °C. The ether extraction procedure was similar to that described by Holdeman et al.[4]. Each pair of analyses with FID and with TCD was performed with the same ether extract. A Tracor (model MT 220) gas chromatograph was used throughout this study. The instrument was equipped with a FID, a TCD, a temperature programming system and dual column oven. It was paired to a recorder Tracor Westronics MT (span: 1 mV/25.5 cm) operated at 2.55 cm/min. 4 U-shaped glass columns (length: 183.0 cm, outer diameter: 0.64 cm, inner diameter: 0.4 cm) packed with 15% SP 1220/1% H_3PO_4 on 100-120 mesh Chromosorb W, acid washed (AW) were used. The SP 1220 stationary phase was obtained from Supelco Inc. Each column end was filled with phosphoric acid treated glass wool and conditioned for 72 h at 185 °C under a

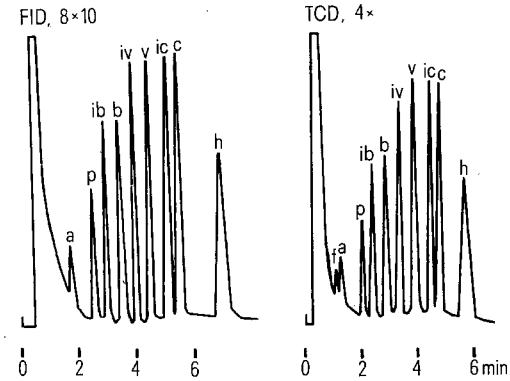

Fig. 1. Chromatograms of VFA standard mixture (1 meq. of each acid/100 cm^3) obtained when analysis conditions with FID and TCD were used. VFA: f, formic; a, acetic; p, propionic; ib, isobutyric; b, butyric; iv, isovaleric; v, valeric; ic, isocaproic; c, caproic; h, heptanoic.

carrier gas flow of 70 cm^3/min. The gases used in this study were zero grade (Liquid Carbonic Canada Ltd, Scarborough, Ontario). Analyses with TCD were performed as described by Hauser and Zabransky[6] oven temperature excepted. Based on preliminary assays, we selected the following temperature program for the oven containing the 4 columns: initial temperature, 125 °C held 1 min; program rate, 40 °C/min; final temperature, 185 °C held 2 min. For analyses using TCD, injector and detector temperatures were respectively 155 °C and 195 °C. The oven temperature was programmed as for analyses with TCD. Carrier gas flow (N_2), hydrogen and air flows were respectively 50, 54 and 470 cm^3/min. An ether extract of the standard VFA mixture containing 1.0 meq/100 cm^3 of each C_1–C_7 acids was used for determining optimal operating conditions. The injection volume was 14.0 µl for analyses with TCD (Scientific Glass Engineering PTY Ltd, 25 ARN syringe) and 1.0 µl for assays with FID (Hamilton 701 N series syringe). During analyses using FID and TCD, inlet pressure of all gases was 2.8 bars. Attenuations used in this study were 8 × 10 for FID and 4 × 1 for TCD.

Figure 1 shows chromatograms of the standard VFA mixture ether extract with FID (left) and with TCD (right). The main difference between the 2 chromatograms was the absence of formic acid (peak f) when FID was used while it was eluted before acetic acid (peak a) when TCD was used. In addition, attenuation required for detection of the VFA mixture was greater with FID. However, injection volume with FID was much smaller than the one used with TCD. The former significantly decreased contamination of the columns. With both types of detectors, the time required for the total resolution of the individual acids, including the cooling operation, did not exceed 8 min. The total resolution time in assays with TCD was shorter due to the higher carrier gas flow rate used (70 cm^3/min) compared to that used in the analyses with FID (50 cm^3/min). Our temperature programmed conditions of VFA analysis either with FID or TCD allowed a sharp separation of the individual VFA. It was particularly interesting for separation of propionic acid (peak p) which may form a shoulder peak with isobutyric acid (peak ib) as previously reported for other stationary phases[6]. Furthermore, the rapid elution of caproic acid (peak c), which was accomplished in less than 6 min, is noteworthy.

Figures 2a, b, c and d illustrate examples of analyses of anaerobic cultures by-products with FID and TCD. Both analyses using FID and TCD are compared. It appeared that the same volatile acids resulting from the fermentation of each 4 cultures were detected with FID or TCD except for the detection of formic acid (peak f) produced by *S. morbillorum* (figure 2b). It can be seen that when propionic acid (peak p) was produced by *P. acnes* or *B. asaccharolyticus* it appeared as a distinct peak on the chromatogram when FID was used (figure 2c, 2d). In all cases, the peaks for acetic, propionic and isobutyric acids were completely resolved. It must be pointed out that these chromatograms of anaerobic bacteria corroborate those shown in the Anaerobe Laboratory Manual (Holdeman et al.)[4] for the corresponding strains cultivated in similar conditions.

Our data show that VFA analysis by GLC was considerably shorter with the oven temperature program we used. Elution of each 10 acids was completed in 8 min. Moreover, since heptanoic acid (peak h) is produced by only a few species, ether extracts chromatography of routinely encountered anaerobic bacteria will not last more than 6 min for each sample, with either analysis conditions with FID or TCD are used. Thus, we have demonstrated that in our operating conditions, the use of SP 1220 stationary phase either with TCD or FID, provides a time saving method for the GLC analysis of VFA produced by anaerobic bacteria. This method can be useful for busy clinical laboratories where large numbers of samples are treated and where oven temperature programming is available. The method wherein described revealed very distinct peaks of each individual volatile fatty acids (C_1–C_7 and isomers) and did not allow misinterpretation either when analysis conditions with FID or TCD were used with the SP 1220 columns. This method permits separation of some methylated non volatile fatty acids (NVFA). However, overlapping of methyl lactate and methyl pyruvate with chloroform necessitates further improvements of the method presented in this paper. Such improvements involving a rapid and simple procedure suitable for the temperature programmed separation of VFA, NVFA, alcohols and ketones and using a single type of gas chromatographic column other than SP1220, were recently described[9].

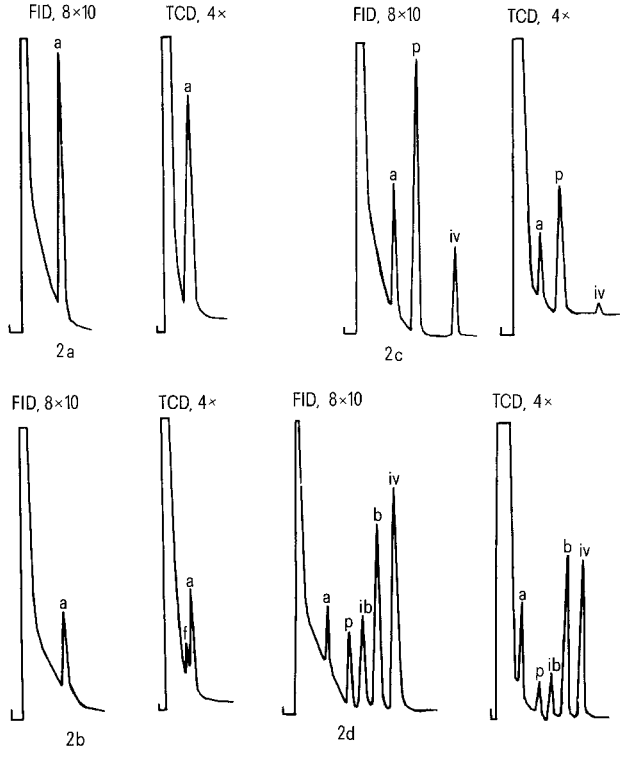

Fig. 2. Chromatograms of ether extract of anaerobes cultures obtained when analysis conditions with FID or TCD were used. VFA: f, formic; a, acetic; p, propionic; ib, isobutyric; b, butyric; iv, isovaleric; *2a C. hystolyticum; 2b S. morbillorum; 2c P. acnes; 2d B. asaccharolyticus.*

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