Note

Detection of non-volatile organic acids by head-space gas chromatography

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Head-space gas chromatography (HSGC) is an established method for the identification of anaerobic bacteria by detection of volatile fermentation products [1]. Alcohols (C_1–C_4) and fatty acids (C_2–C_8) can be analysed quickly and reproducibly. High accuracy is obtained by automation of the head-space sampling. In our laboratory we have been using this method for the identification of anaerobic spore-forming (*Clostridia*) and other pathogenic bacteria. Anaerobic bacteria can generally be identified as to genus, and sometimes as to species.

However, non-volatile fermentation products such as lactate and succinate cannot be detected by HSGC because of their high boiling points [2]. In general, these acids are extracted, methylated and then injected manually or by an auto-sampler on the GC column [3–5].

This paper describes a method in which the methylation takes place in the head-space vial and the organic acids are converted into their volatile methyl esters or dimethyl esters and injected by the autosampler

EXPERIMENTAL

Gas chromatography

All analyses were carried out with a Perkin-Elmer gas chromatograph Sigma 2000 with a head-space autosampler HS-100 (Bodenseewerk Perkin-Elmer, Über-lingen, F.R.G.). The column was a Restek-StabilwaxDA capillary column (Am-chro, Sulzbach/Taunus, F.R.G.), 30 m × 0.32 mm I.D. with a film thickness of 0.25 μm. The detector was a flame ionization detector.

The sampling conditions were as follows: vial temperature, 120°C; transfer line temperature, 150°C; thermostatting time, 20 min; pressurization time, 0.2 min; injection time, 0.08 min; withdrawal time, 0.2 min; pressure of the high pressure sampling was 250 kPa.

The column temperature was held initially at 70°C for 3 min, then raised at 6°C/min to 120°C, then increased to 200°C at 30°C/min. The run time was 14 min, and the detector temperature was 250°C. The carrier gas was nitrogen (3 ml/min).

Chromatographic data were processed with a Perkin-Elmer LCI-100 laboratory computing integrator, which was connected to an IBM-XT computer.

Chemicals and solutions

The standard solution contained pyruvate (10 mM, pyruvic acid sodium salt), lactate (10 mM, L-lactic acid sodium salt), oxalate (5 mM), methylmalonate (5 mM), malonate (5 mM), fumarate (5 mM), and succinate (5 mM) (all from Fluka, Buchs, Switzerland).

A 20-μl aliquot of the sample was pipetted into a head-space vial, and 10 μl of a saturated NaHSO$_4$ solution and 10 μl of methanol were added. The acids are converted in the vial into the volatile methyl esters. NaHSO$_4$ causes a "salting-out" effect and gives an acidic pH, which is necessary for esterification. The vial temperature for this reaction was maintained at 120°C for 20 min.

RESULTS

A chromatogram of the standard solution is shown in Fig. 1. The large peak at the beginning of the chromatogram corresponds to methanol. All components were eluted within 10 min.

The accuracy of the method was tested at the concentrations indicated in Table I. The results are the means of five analyses ($n = 5$). A t-value of less than 2.78 means a low difference between the sample concentration and the result of the analysis [6].

The correlation coefficients were estimated by analysing five solutions containing 10, 25, 50, 75 and 100% of the concentrations of the components in the calibration standard. Linear regression analysis gave correlation coefficients between 0.997 140 and 0.999 874 for the compounds under study

The yield of methylation was determined by analysing a solution containing 10 mM methyl-DL-lactate (Fluka) and 5 mM dimethyl succinate (Fluka). The yields were 99.8% for lactate and 95.5% for succinate.

A chromatogram of a *Bacteroides fragilis* culture is shown in Fig. 2. Lactic acid (1.07 mg/ml) and succinic acid (1.02 mg/ml) were detected as main fermentation products of this *Bacteroides* strain.

Fig. 3 shows a chromatogram of a BHK tissue culture sample (BHK = baby hamster kidney). Lactic acid (4.2 mg/ml) is a byproduct of the catabolism of eukaryotic cells in tissue cultures.

Thus the method can be used for HSGC analysis of pyruvic, lactic, oxalic, methylmalonic, malonic, fumaric and succinic acids. It is a simple way to detect rapidly and accurately these non-volatile organic acids in any liquid samples. A time-consuming derivatization procedure is not necessary
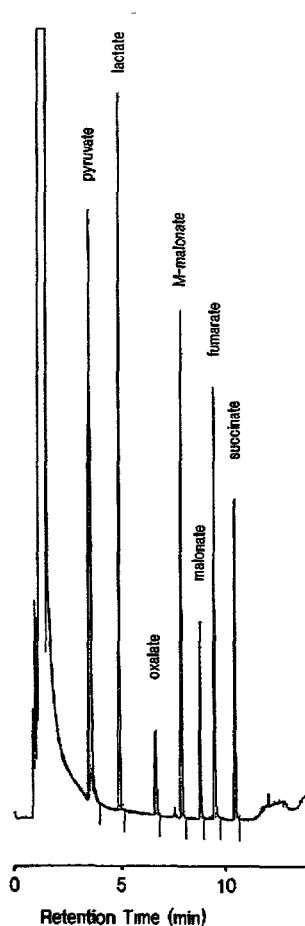
Fig. 1. Chromatogram of a standard solution containing pyruvate and lactate (10 mM each) and oxalate, methylmalonate, malonate, fumarate and succinate (5 mM each)

TABLE I

ACCURACY OF THE METHOD

The accuracy was estimated by analysing five samples containing 75% of the concentrations of the components in the calibration standard.

Component	Concentration (mg/ml)	Recovery (mg/ml)	S.D.		t-Value
			$\pm s$	$\pm s$ (%)	
Pyruvate	0.66075	0.6404	0.0145	2 26	3 11
Lactate	0.67575	0.6879	0.0121	1 76	2 25
Oxalate	0 3375	0.3447	0 0086	2 49	1 87
Methylmalonate	0.4425	0.4310	0 0161	3 74	1.60
Malonate	0 3900	0.3961	0.0119	3 00	1.15
Fumarate	0.4350	0 4243	0 0167	3.94	1.43
Succinate	0.4425	0.4487	0 0135	3.01	1 03

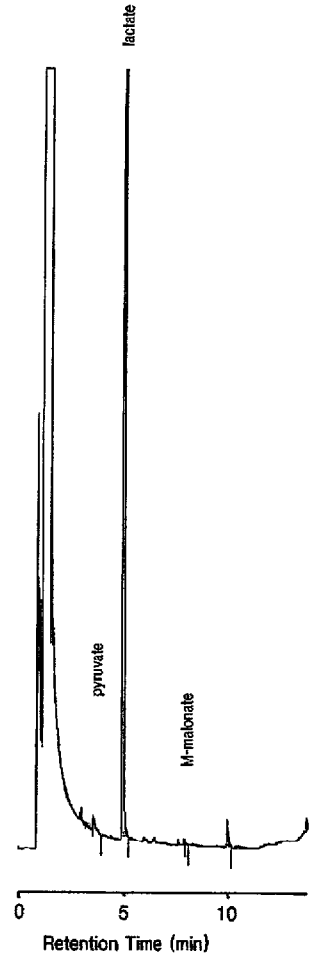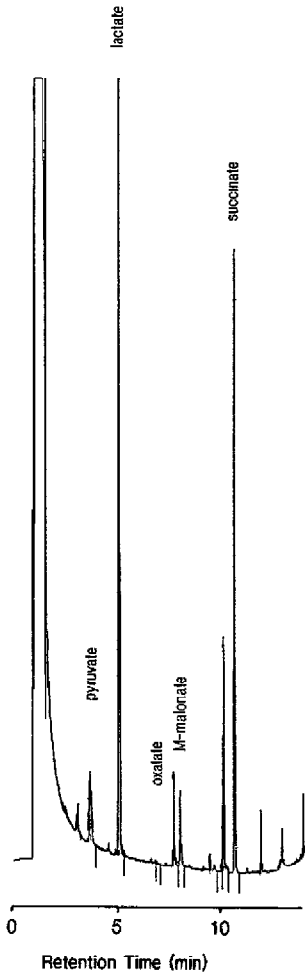Fig. 2. Chromatogram of a *Bacteroides fragilis* fermentation broth

Fig 3 Chromatogram of a BHK tissue culture sample

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