Analysis of Bacterial Fatty Acids by Flow Modulated Comprehensive Two Dimensional Gas Chromatography with Parallel Flame Ionization Detector / Mass Spectrometry

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INTRODUCTION

A commercially available flow modulated GC×GC system was tested and optimized for the analysis of bacterial fatty acids (as methyl esters). The system configuration included parallel MS and FID detection. The results are compared to data obtained on a thermal modulation system.

EXPERIMENTAL

A bacterial acid methyl ester (BAME) solution in methyl caproate obtained from Supelco was used as a reference sample. A *Stenotrophomonas maltophilia* bacteria sample was prepared using the Sherlock MIDI standard operating procedure (M. Sasser, MIDI Technical Note 101, 1990, see www.midi-inc.com).

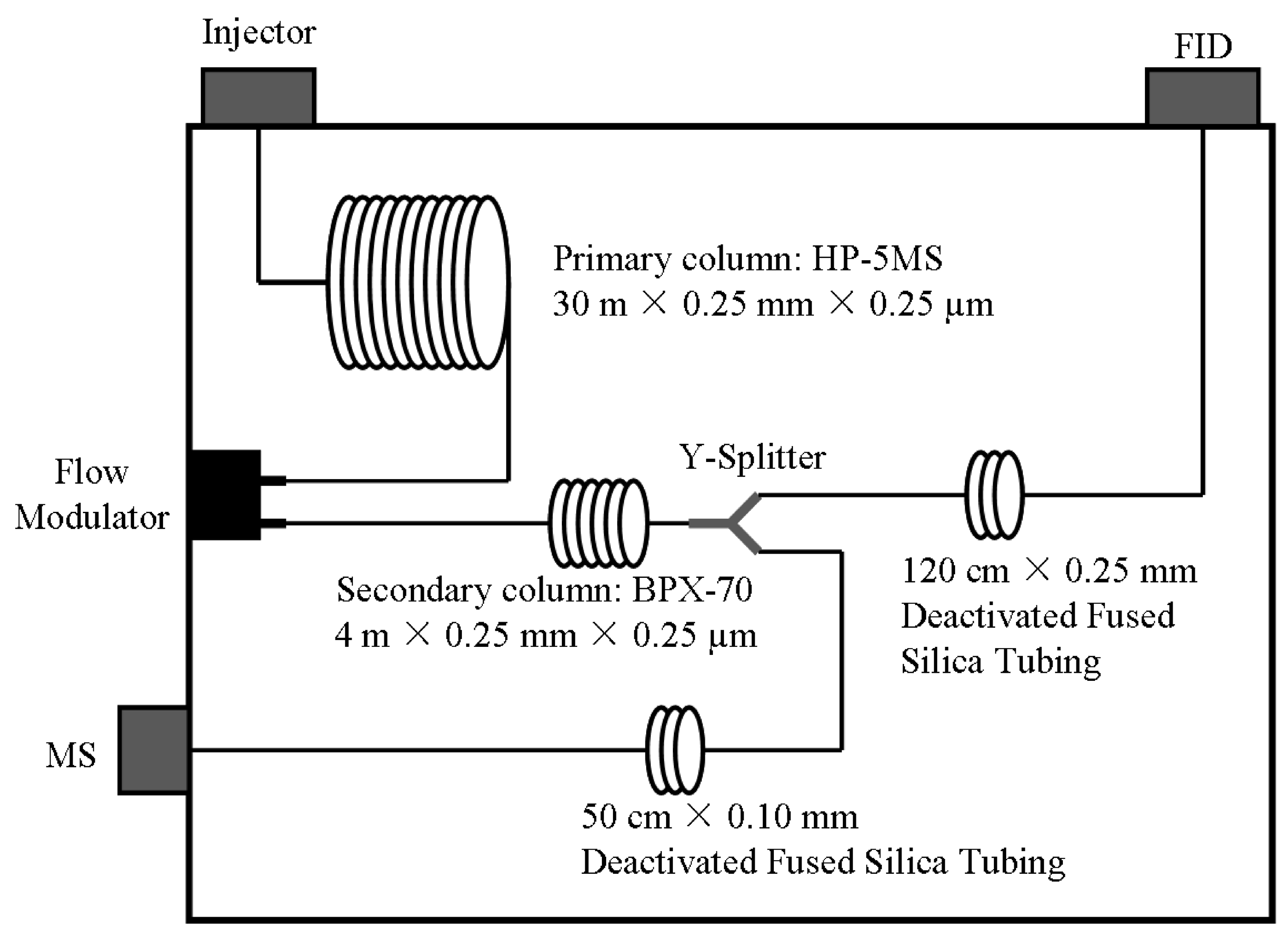

Fig.1 A schematic of the flow modulated GC×GC with FID and MS

Analytical parameters

Instrument: Agilent 7890A GC & 5975 MSD
Inlet: SSL at 250 °C, 1 µL, split ratio 10:1
Carrier gas: Hydrogen, constant flow
First dimension column: HP-5MS 30 m x 0.25 mm x 0.25 µm
^{1}D gas flow: 0.6 mL/min
Second dimension column: BPX-70 4 m x 0.25 mm x 0.25 µm
^{2}D gas flow: 25 mL/min
Modulation time: 2 s; *Sample time*: 1.9 s
Oven: 100 °C (2 min) - 2 °C /min - 240 °C (10 min)
Detection: FID and MS (scan range *m/z* 40 - 430; 20 scans/s)

OPTIMIZATION

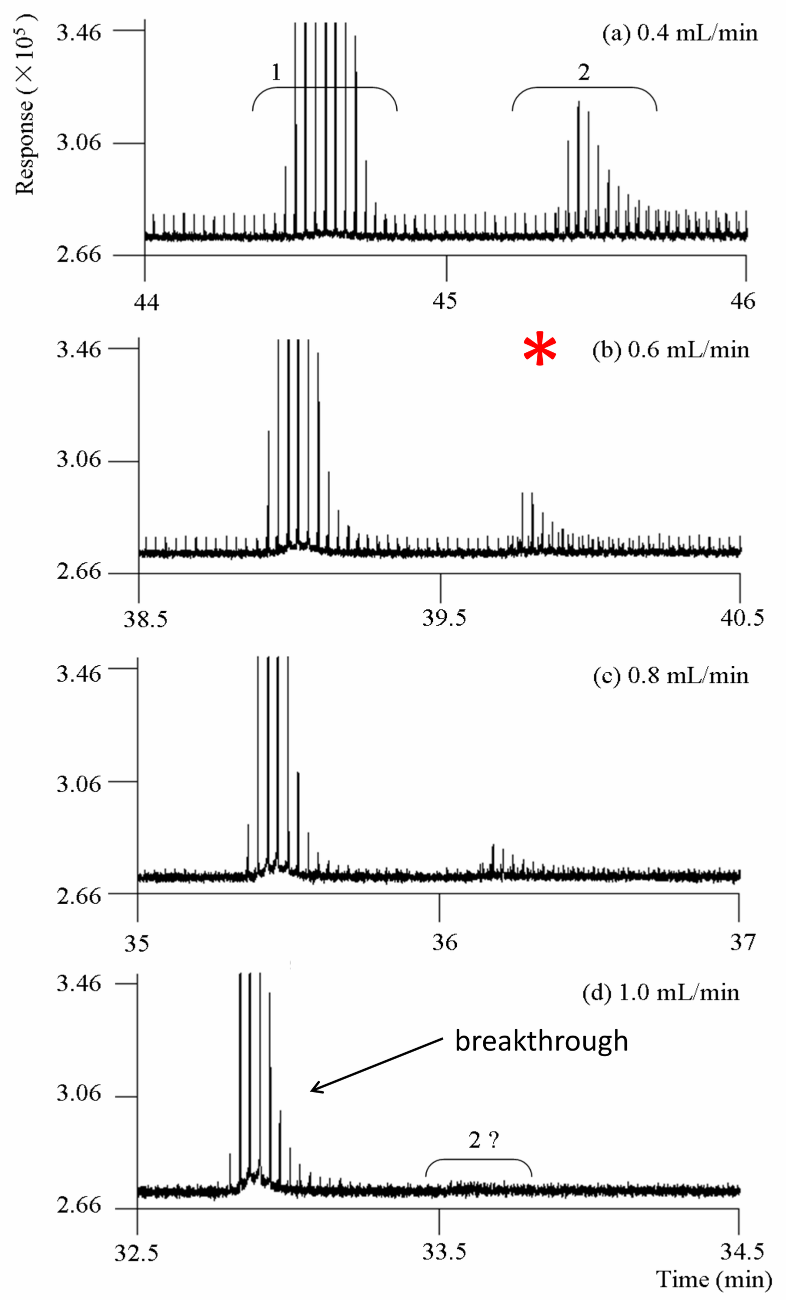

Fig. 2 The modulated chromatograms at different ^{1}D flow rates. 1. 13:0; 2. 12:0 2OH.

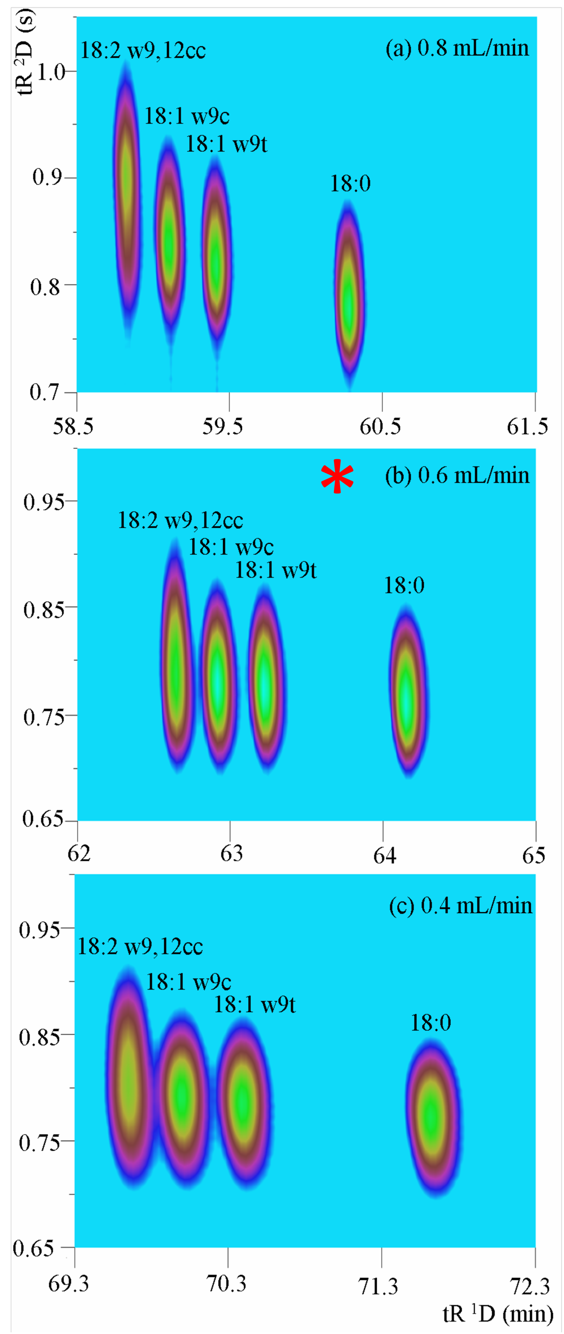

Fig. 3 The GC×GC plots of the C18 fatty acid elution area at different ^{1}D flow rates

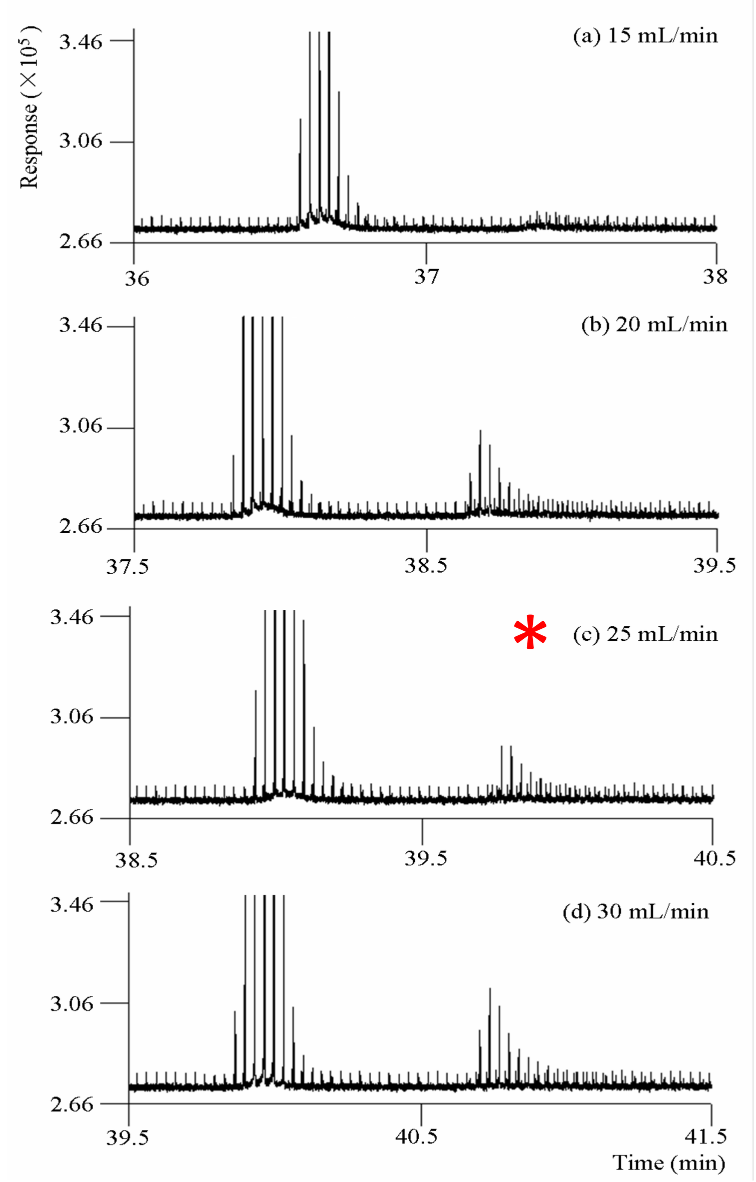

Fig. 4 The influence of the ^{2}D flow

*** Optimal conditions:**

^{1}D gas flow: 0.6 mL/min
^{2}D gas flow: 25 mL/min
Modulation time: 2 s
Sample time: 1.9 s

RESULTS

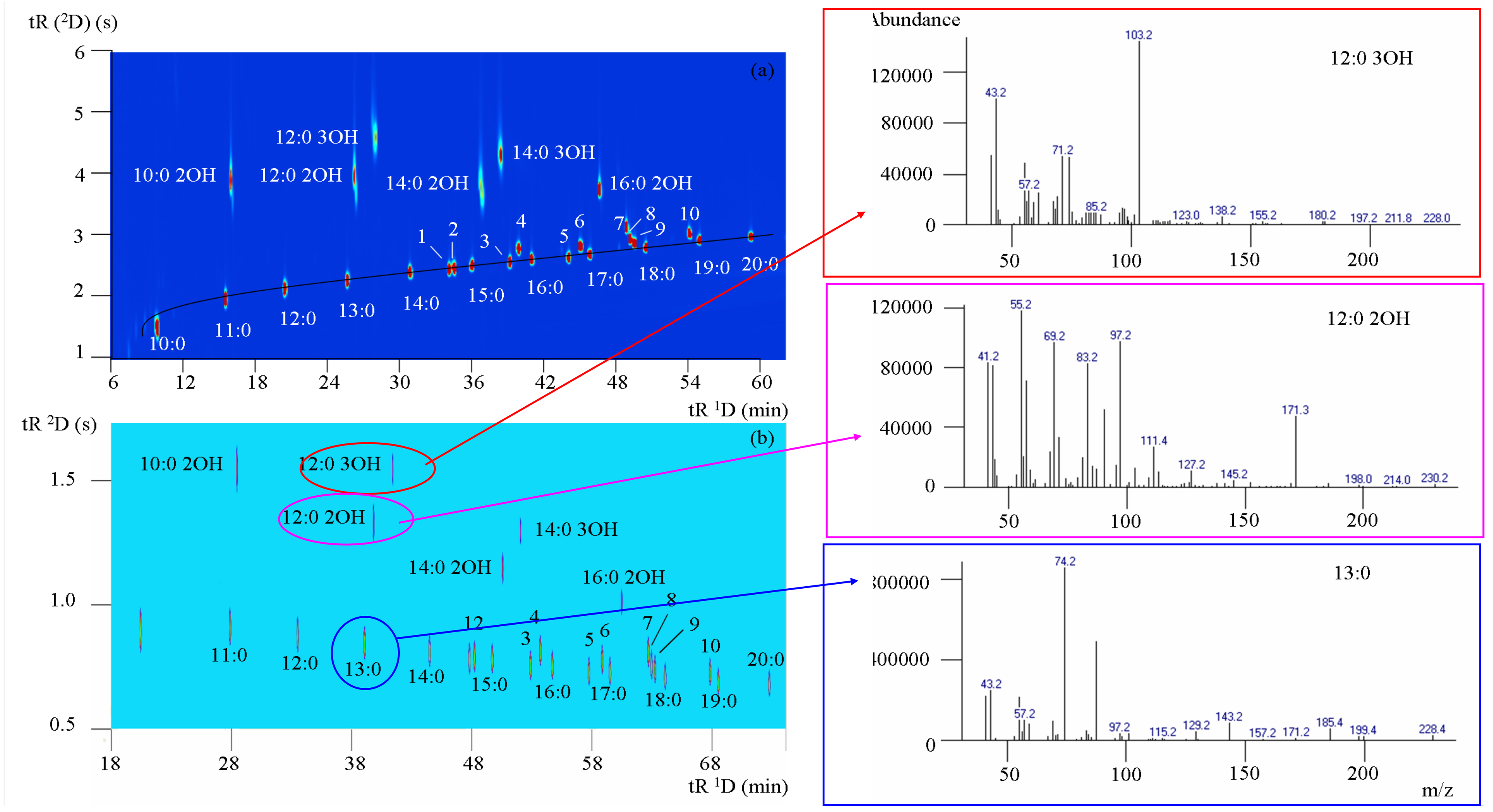

Fig. 5 GC×GC plots of a BAME reference sample obtained by thermal modulation (a) and flow modulation (b) and mass spectra of typical solutes in BAMEs standards

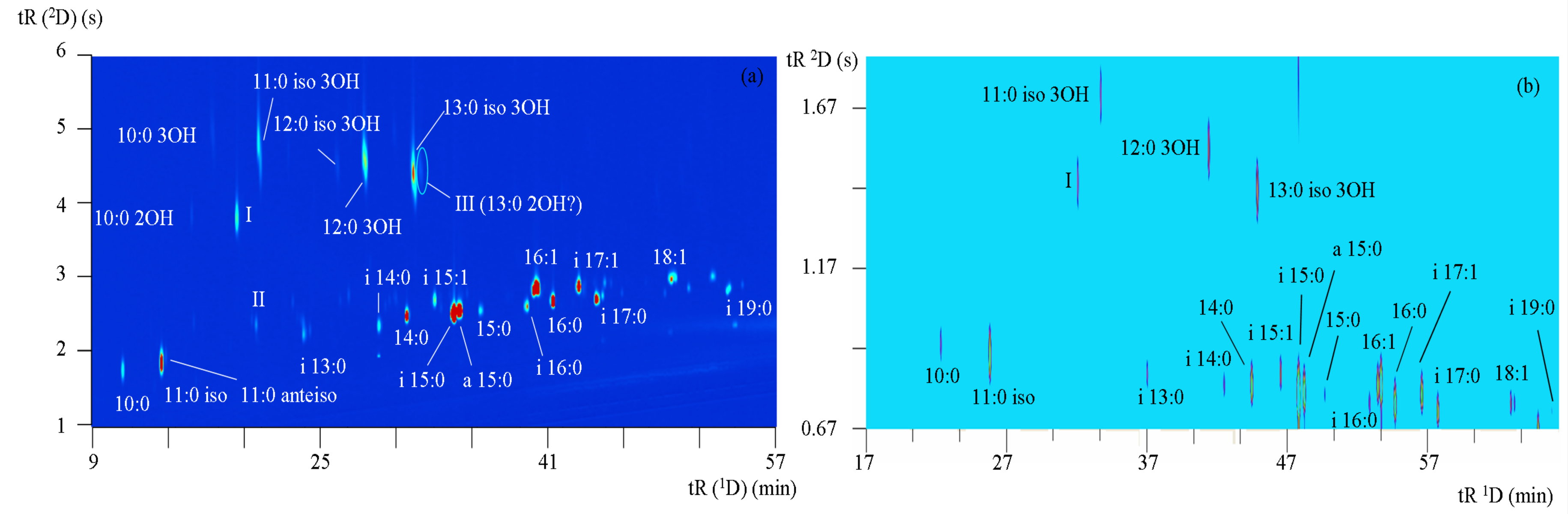

Fig. 6 GC×GC plots of BAMEs from *S. maltophilia* by thermal (a) and flow modulated GC×GC (b)

CONCLUSIONS

The GC×GC plots obtained for a reference sample of bacterial fatty acid esters and a bacteria sample (*S. maltophilia*) were very similar to those obtained by thermal modulated GC×GC. The GC×GC approach is especially interesting in detecting the presence of hydroxy fatty acids. The parallel FID/MS set-up is useful since the MS allows identification and confirmation, while the FID allows comparison of the relative fatty acid composition with existing databases (Table 1).

Table 1. Relative composition of BAMEs in *S. maltophilia*

Compound	Peak I (min)	Peak II (sec)	volume (%)	MIDI data (%)
10:0	22.30	0.94	0.78	0.76
11:0 iso	25.80	0.90	4.82	4.37
I (unknown)	32.07	1.44	1.55	2.02
11:0 iso 3OH	33.70	1.71	1.74	2.22
i 13:0	37.00	0.85	0.78	0.50
12:0 3OH	41.40	1.54	3.04	3.85
i 14:0	42.50	0.81	0.58	0.62
14:0	44.47	0.80	4.10	3.04
13:0 iso 3OH	44.83	1.42	3.38	4.93
i 15:1	46.50	0.84	1.33	0.91
i 15:0	47.77	0.77	44.15	35.24
a 15:0	48.20	0.78	6.12	9.29
15:0	49.67	0.78	0.35	0.45
i 16:0	52.83	0.75	0.57	1.05
16:1 w9c	53.43	0.81	2.82	2.79
16:1 w7c	53.67	0.81	11.58	10.74
16:0	54.67	0.75	5.03	6.35
i 17:1 w9c	56.57	0.78	3.76	4.18
i 17:0	57.70	0.72	2.22	3.22
18:1 w9c	62.90	0.75	0.83	1.14
18:1 w7c	63.17	0.75	0.47	0.63