Fast Temperature-Programmed Gas Chromatography–Mass Spectrometry for Food Analysis

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Abstract

Fast temperature-programmed gas chromatography (FTGC) is evaluated. A modified capillary column allowing temperature-program rates as high as 20°C/s is operated with mass spectrometric detection (MSD). FTGC methods are developed for the analysis of off-flavors in water, derivatized sugars, and fatty acid methyl esters. Liquid injection and solid-phase microextraction are evaluated. Although the resolution can be somewhat lower than analysis with a traditional column, time savings are significant. Analysis times range from 2 to 4.5 min compared with 20 to 60 min for conventional methods. MSD allows for the analysis of some coeluting peaks, which allows for quantitation even when peaks coelute.

Introduction

Because of the large numbers of samples and fast turnaround times needed in today's laboratories, rapid and highly sensitive analytical methods are required. Capillary gas chromatography (GC) usually requires analysis times of tens of minutes to more than an hour. For example, sugar analysis requires 31 min (1), fatty acid analysis for microbial identification requires 23.3 min (2), and the analysis of fatty acid methyl esters (FAMEs) in foods requires 66 min (3).

The most common technique used to decrease analysis time in GC is increasing the column temperature during the run. Most commercially available chromatographic ovens are limited to temperature-program rates of 20 to 40°C/min. Encasing a short length of capillary column (5 or 10 m) in a resistance heated stainless steel tube allows for much faster temperature-program rates (as high as 1200°C/min). This equipment is commercially available (Thermo-Orion, Beverly, MA) and can be equipped with any type and dimension of capillary column chemistries. Dallüge et al. (4) described this technique for the analysis of standard mixtures of alkanes, pesticides, and polyaromatic hydrocarbons analyzed by GC using a flame ionization detector.

In this study, we focused on the advantages and disadvantages of fast temperature-programmed GC (FTGC) with a quadrupole mass-selective detector (MSD). The problem of using a short column on the vacuum chamber of the MSD was explored using an alkane standard. In addition, three potential applications were discussed: the solid-phase microextraction (SPME) analysis of flavors, sugar syrup analysis, and fatty acids in food and bacteria.

Flavor analysis is difficult because of the complexity and low

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Table I. Chromatographic Conditions*

Line	Experiment	Linear Velocity	Split ratio	Splitless time (min)	Initial temp. (°C)	Initial time (min)	Rate 1 (°C/min)	Temp. 1 (°C)	Rate 2 (°C/min)	Final temp. (°C)	Final time (min)	Run time (min)
1	Resolution	varied	40:1	0	50	0	120	300	–	–	0.42	2.5
2	SPME	19.2	40:1	1	50	1	120	300	–	–	0.42	3.5
3	Alkane	19.2	12:1	0.25	35	0.5	180	260	–	–	0.75	2.0
4	Fast sugar	22.3	40:1	0.2	100	0	120	310	–	–	1.25	3.0
5	Standard sugar	22.3	40:1	0.2	100	4	10	310	–	–	10.00	35.00
6	FAME	19.2	50:1	0.2	50	0	60	260	–	–	1.50	3.5
7	Standard MIS	22.3	40:1	0.2	170	0	5	270	30	310	2.00	23.3
8	Fast MIS	22.3	40:1	0.2	80	0	120	310	–	–	0.72	2.6

* The liquid phase was 95% dimethyl–5% diphenyl polysiloxane except line 6. The FAME analysis was performed on a 90% biscyanopropyl–10% cyanopropylphenyl polysiloxane column.

concentration of flavor chemicals in food products. The routine monitoring of a food-processing assembly line would require a simple, rapid, reliable, and inexpensive technique. SPME (5) reduces the complexity and cost of analysis. Combining SPME with FTGC should result in a useful method.

Sucrose is lost through degradation in aqueous acid or alkaline solutions during sugar refining and manufacturing (6). Such losses could be monitored by analyzing for stable degradation products (7). Monitoring the refining process with a rapid and robust method would facilitate sugar refining.

Mandatory food labels require that total, saturated, and polyunsaturated fat content be listed. In the near future, the *trans* fat content may also be required (8). A rapid method to analyze all forms of fat would be useful to food processors.

The fatty acids in microbial cell walls are species specific. Results from the fatty acid analysis of a microbial colony are matched with a library of profiles to identify the species (2). This procedure is much faster than identifying microbes by growing them on differential media and performing chemical analyses. The use of FTGC would enhance the time savings.

Table II. The Effect of Linear Velocity on Vacuum Chamber Pressure, Resolution, and Sensitivity*

Head pressure (psi)	RT (min)	Peak area	Resolution	Chamber pressure (× 10^{-5} torr)	Linear velocity (cm/s)
2	1.49	1,409,288	2.9	2.1	19.2
5	1.38	1,006,933	3.7	3.0	22.3
10	1.25	698,457	3.9	4.7	32.2
15	1.15	484,730	4.5	6.6	37.9
20	1.08	329,142	5.2	8.8	36.9
25	1.02	258,011	5.6	11.0	41.8

* Resolution was calculated between undecane and dodecane. Chromatographic conditions are given in Table I, line 1.

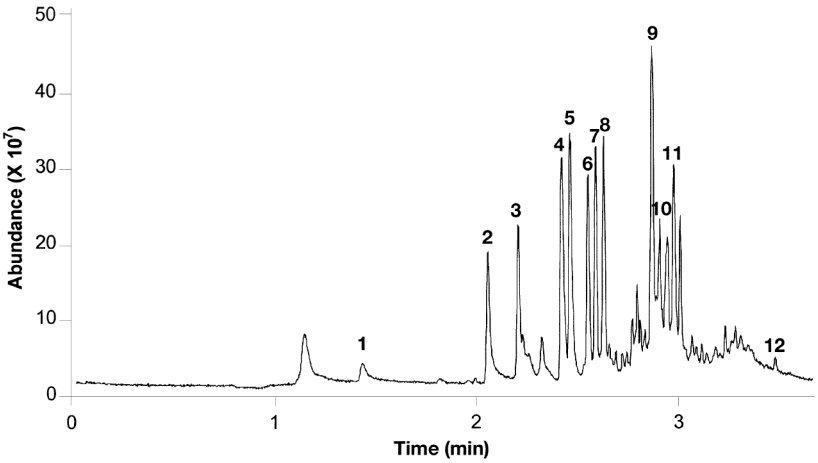

Figure 1. SPME analysis of a 40-µg/L solution of 12 analytes in water: (1) pentanal, (2) heptanal, (3) heptanol, (4) 2-nonanone, (5) methyl octanoate, (6) isoborneol, (7) 2-methylisoborneol, (8) β-cyclocitral, (9) geosmin, (10) α-humulene, (11) nerolidol, and (12) methyl octadecanoate. Chromatographic conditions are listed in Table I, line 2.

Experimental

Reagents

Solvents were obtained from Baker Chemical Company (Phillipsburg, NJ). Methyl esters and flavor standards were purchased from Sigma-Aldrich (St. Louis, MO). The Microbial Identification System (MIS) calibration mix was purchased from Microbial ID, Inc. (Newark, DE). The 37-component FAME mix and RM-6 AOCS Animal and Vegetable Reference Mix were purchased from Supelco, Inc. (Bellefonte, PA).

Equipment

A Hewlett Packard 5890 Series II GC was equipped with a split/splitless injection port, a 7673A autosampler, and a 5970 MSD controlled by ChemStation software (Agilent Technologies, Palo Alto, CA). Hydrogen was used as the carrier gas. For SPME analyses, the injection port was equipped with a 0.75-mm-i.d. liner (Supelco). For injection analyses, a 4-mm-i.d. glass wool packed liner was used (Agilent Technologies). The GC was equipped with an EZ-Flash temperature-programmable column (Orion Research, Inc., Beverly, MA). Two column phases were used: an RTX-5 (95% dimethyl–5% diphenyl polysiloxane) and an RTX-2330 (90% biscyanopropyl–10% phenylcyanopropyl polysiloxane). Each column was 10 m long with a 0.25-mm i.d. and a 0.2-µm phase thickness. Standard temperature-programming rate experiments were performed using a 30-m × 0.25-mm column with a 5% phenyl–methylsiloxane phase (ZB-5, Phenomenex, Torrance, CA). Chromatographic conditions are listed in Table I. SPME fibers (100-µm polydimethylsiloxane and divinylbenzene–carboxen in polydimethylsiloxane) were obtained from Supelco.

Resolution and sensitivity

Peak resolution and linear range of the FTGC column coupled with the HP5970 MSD were studied using 1-µL injections of a 100-mg/L solution of *n*-alkanes (from heptane to eicosane) in hexane. The column used was a 10-m RTX-5. The chromatographic conditions are given in Table I, line 1. The MSD was scanned from 50 to 385 amu. The column head pressure was varied and the split ratio adjusted to 40:1. The MSD vacuum chamber pressure was measured with an ion gauge and recorded for each head pressure. The same mixture was analyzed using a 30-m capillary column and a 10°C/min temperature-programming rate (Table I, line 5).

SPME and flavor

The 100-mg/L *n*-alkane solution was used to study the use of SPME in conjunction with fast temperature programming. A 1-µL sample was combined with 6 mL of water, 1.9 g NaCl, and a magnetic stirbar in a sealed vial with a 12-mL capacity. The vial was stirred in a water bath at 40°C while a SPME fiber (100 µm polydimethylsiloxane) was exposed to the headspace for 15 min. The fiber was desorbed in the injection port of the GC equipped with a 0.75-mm-i.d. liner and held at

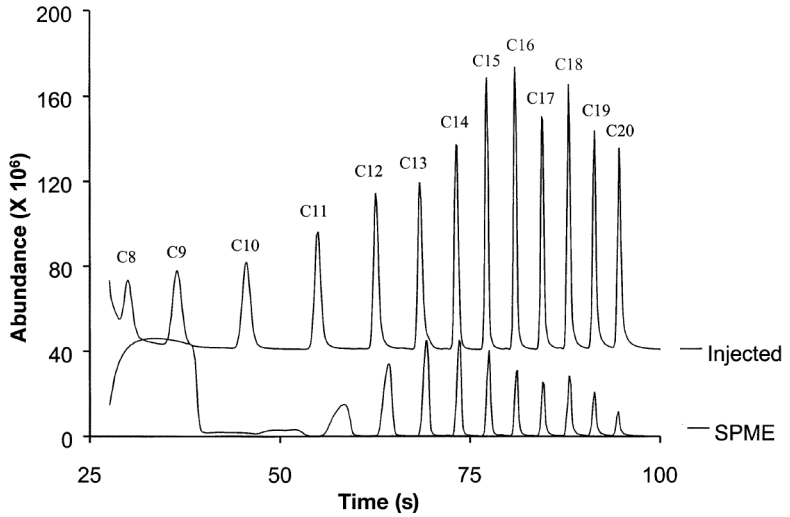

Figure 2. A 1-µL sample of a 100-mg/L solution of C_7–C_{20} *n*-alkanes analyzed by injection and SPME. Data from the injection are offset by 40 x 10^6 abundance units. Chromatographic conditions are listed in Table I, line 3.

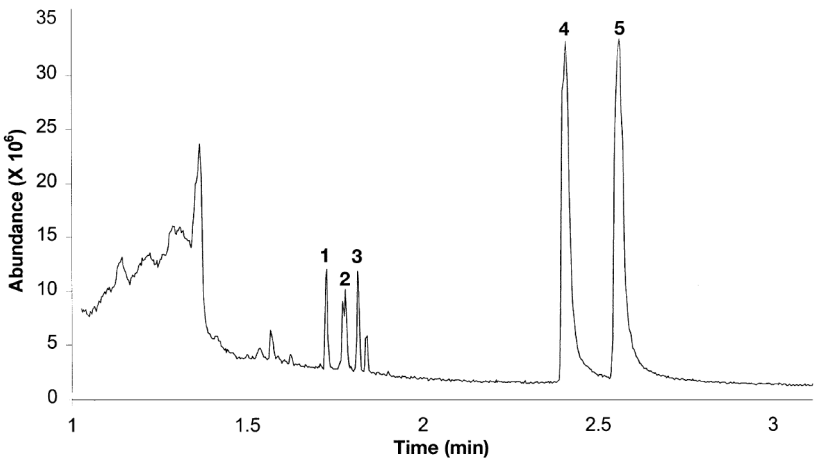

Figure 3. A 1-µL sample of a sugar syrup standard analyzed by FTGC–MSD: (1) methyl α-D-glucopyranoside, (2) α + β fructose, (3) α + β glucose, (4) sucrose, and (5) trehalose. Chromatographic conditions are listed in Table I, line 4.

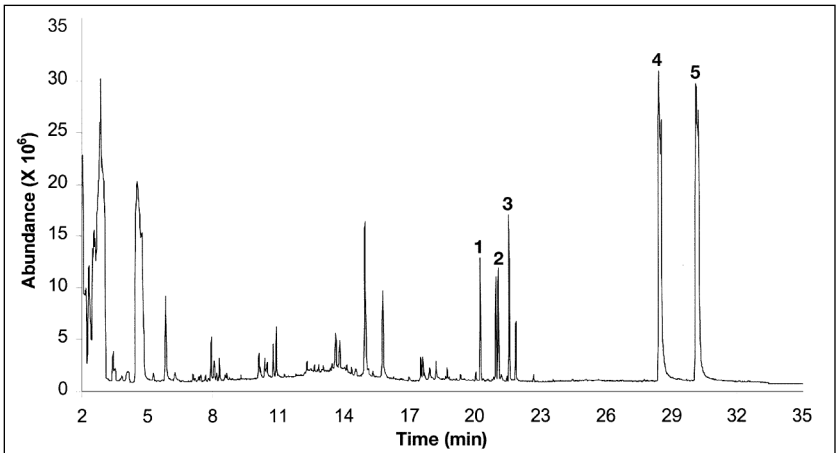

Figure 4. A 1-µL sample of a sugar syrup standard analyzed by GC–MSD: (1) methyl α-D-glucopyranoside, (2) α + β fructose, (3) α + β glucose, (4) sucrose, and (5) trehalose. Chromatographic conditions are listed in Table I, line 5.

250°C, and the desorbed alkanes were analyzed (Table I, line 3). A 1-µL aliquot was analyzed by injection under the same chromatographic conditions. The MSD was scanned from 50 to 300 amu.

Solutions containing 12 chemicals commonly found in food and drinking water were analyzed using SPME. A 6-mL solution was placed in a 10-mL vial, and a magnetic stirbar and 1.9 g NaCl were added. The vial was sealed and placed in a water bath at 65°C. The SPME fiber (divinylbenzene–carboxen in polydimethylsiloxane) was exposed to the headspace for 15 min while the contents were stirred. Three vials were processed simultaneously at staggered intervals so that one fiber was ready for desorption every 5 min. Each fiber was desorbed in the injection port of the GC for 1.0 min at 270°C, and the desorbed volatiles were analyzed (Table I, line 2). The MSD was scanned from 33 to 300 amu.

Sugar analysis

A sugar standard was analyzed according to the 1998 ICUMSA methods (1). Aliquots of sugar solutions were oximated with hydroxylamine hydrochloride in pyridine. The oximated solutions were then silylated with hexamethyldisiloxane and analyzed by FTGC–MSD (Table I, line 4) and GC–MSD (Table I, line 5). The MSD was scanned from 70 to 500 amu.

Fatty acid analysis

A mixture of 37 FAMEs was analyzed by FTGC–MSD (Table I, line 6). Another mixture of FAMEs (FAME Mix RM-6) was diluted with heptane to yield solutions with concentrations of methyl tetradecanoate (ranging from 1.995 to 1995 mg/L) and methyl octadecatrienoate (ranging from 3.001 to 3001 mg/L). The MIS calibration mix (a mixture of saturated- and hydroxy-FAMEs) was analyzed by GC–MSD using the standard conditions for bacterial identification (2) (Table I, line 7) and FTGC–MSD (Table I, line 8). The MSD was operated in selected ion monitoring mode. The ions selected were 69, 74, 83, 129, 103, 143, 199, and 227 amu.

Results and Discussion

Resolution and sensitivity

In order to demonstrate the relationship between resolution and sensitivity on an MSD equipped with an FTGC column, we analyzed an alkane solution with six different head pressures. The results are presented in Table II. Resolution improves as the linear velocity approaches the

optimum value for hydrogen carrier gas (40 cm/s); but as the vacuum chamber pressure increases, sensitivity decreases. A higher vacuum chamber pressure results in an increasing number of collisions between ions and carrier gas molecules, which decreases the number of ions reaching the detector. A tradeoff between resolution and sensitivity must be made when developing a method for FTGC–MSD. For maximum sensitivity, a velocity considerably less than optimal might be used. For this study, a head pressure of 5 psi corresponding with a linear velocity of 22 cm/s was chosen as a compromise between sensitivity and resolution.

Table III. Comparison of Calibration Standards for the MIS Analyzed by FTGC–MSD and GC–MSD*

	FTGC		GC	
Ester	RT (min)	Area	RT (min)	Area
C9:0	1.25	620	3.5	385
C10:0	1.36	771	4.4	726
C11:0	1.46	568	5.6	450
C10:0 2OH	1.48	126	5.8	141
C10:0 3OH	1.50	17	6.2	40
C12:0	1.56	950	7.0	740
C13:0	1.65	617	8.7	521
C14:0	1.75	944	10.5	903
C15:0	1.83	583	12.4	556
C14:0 2OH	1.86	88	12.8	110
C14:0 3OH	1.88	24	13.3	15
C16:0	1.92	1000	14.3	1000
C17:0	2.00	565	16.2	609
C16:0 2OH	2.02	92	16.7	105
C18:0	2.08	852	18.1	997
C19:0	2.16	594	19.9	609
C20:0	2.25	914	21.3	812

* Chromatographic conditions are given in Table I, line 7 (FTGC) and line 8 (GC).

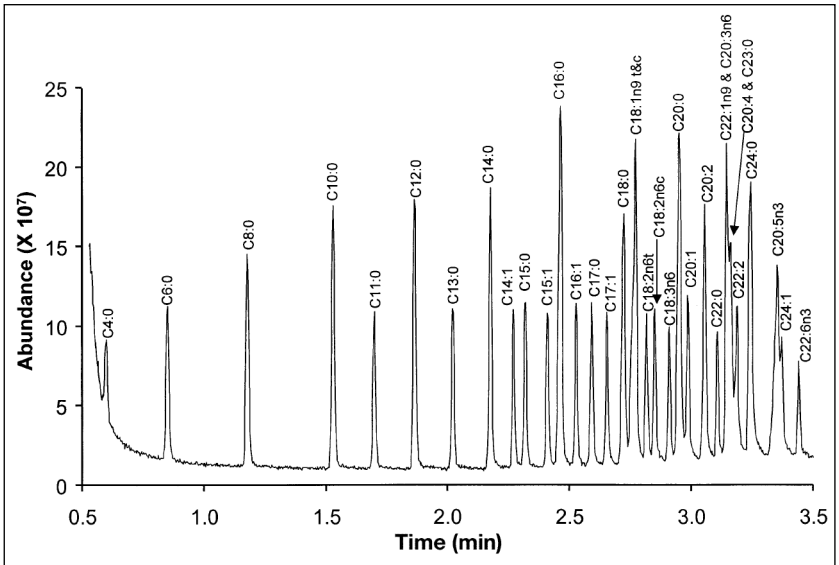

Figure 5. Total ion chromatogram from the FTGC–MSD analysis of 1 µL of a mixture of 37 FAMEs of varying concentrations. Chromatographic conditions are listed in Table I, line 6. Codes used to identify the peaks are defined in Table IV.

There is nothing special about the columns used in FTGC, other than the short length. It is the ability to use much higher temperature-programming rates that allow faster chromatographic runs. When the alkane solution was analyzed using a traditional GC column operated at 41 cm/s linear velocity, the resolution between undecane and dodecane was 6.7. When this is compared with the value of 5.6 for the same pair listed in Table II for the same linear velocity, it can be seen that the short length of the FTGC column has a negative impact on resolution.

SPME

A chromatogram from the analysis of a mixture of compounds found in food and water is shown in Figure 1. The unlabeled peaks are contaminants from the SPME fiber and the water and septum bleed. All 12 compounds of interest eluted in less than 3.5 min. The resolution between isoborneol (RT = 2.551) and methylisoborneol (RT = 2.590) was 0.907. When the same experiment was run on a 30-m capillary column with a 25°C/min temperature-program rate and a 40-cm/s linear velocity, the resolution between the same pair was 2.196.

The widths of the pentanal (0.041 min at half height) and heptanal peaks (0.030) were wider than subsequent peaks (which range from 0.020 to 0.011). When analytes were desorbed from a SPME fiber, they were focused on the head of the column while the temperature was held at a relatively low level. The temperature program started when desorption ended. The shorter column used in FTGC provided less focusing. This is shown more dramatically in Figure 2. A mixture of alkanes was analyzed by injection (upper trace) and SPME (lower trace). Injection allows for the separation of compounds with boiling points higher than that of heptane. Because SPME requires focusing compounds at the head of the column, acceptable peak shapes were observed only for compounds with boiling points above that of undecane. At the temperatures used in this study (shown in Table I, line 2), compounds with lower boiling points are not focused sufficiently during the desorption step. They coelute with hexane. Starting the temperature program at a lower temperature might compensate, but the run would be longer. The use of a cryotrap could resolve the earlier eluting peaks.

In general, SPME requires extraction times ranging from 5 to 60 min (12). The optimal extraction time for these compounds is 15 min. Because the cycle time for the chromatograph is 5 min (3.7 min run time plus 1.3 min cool down time), 3 samples were extracted at staggered intervals using 3 fibers in manual fiber holders. Because SPME autosamplers are restricted to extracting samples sequentially, the advantage of FTGC will not be realized for autosampler-equipped GCs if the run times are less than the extraction times.

Sugar analysis

Figures 3 and 4 show the chromatograms from the FTGC–MSD and GC–MSD analysis of the standard sugar mixture. Separation of the three sugars (glucose, fructose, and sucrose) was achieved in

less than 3 min by FTGC, compared with 23 min for Standard Method GS7/4-22. The resolution was lower when using FTGC. The α and β isomers of fructose were baseline resolved by GC, but not by FTGC. However, GS7/4-22 specifies that the geometric isomers be summed together, thus this loss of resolution is not a problem with this analysis.

Fatty acid analysis

A chromatogram from the analysis of the 37-component FAME mix is shown in Figure 5. The separation of several compounds was incomplete. Coelutions were found with the *cis*- and *trans*-isomers of C18:1, C22:1n9–C20:3n6, and C20:4–C23:0. Several other peak pairs were not completely separated. The MSD can resolve coelutions involving different compounds by monitoring different ions for each compound, but it performs poorly in separating positional isomers such as *cis*- and *trans*-fatty acids because the compounds have nearly identical mass spectra.

Diluted solutions of an RM-6 mix (a mixture of FAMEs similar to the fatty acid distribution found in lard, beef, and mutton tallow and palm oil) were analyzed in order to study linearity and sensitivity. At a split ratio of 50:1 and a concentration of 1995 mg/L, the methyl tetradecanoate peak displayed fronting, indicating column overload. Under the same conditions, the lowest level detected was 0.1995 mg/L (signal-to-noise ratio = 4.86). The correlation coefficient for the concentration range between 0.1995 and 199.5 mg/L was 0.9994, thus indicating good linearity over three orders of magnitude. Using a column with a thicker phase could increase this range, although resolution might be reduced. The column used for this work had a 0.2-µm phase thickness.

Results from the analysis of the bacterial acid methyl ester calibration standard are presented in Table III. The chromatograms appear in Figures 6 and 7. Area counts listed in Table III were normalized to the C16:0 peak, showing good agreement between methods. The standard run time was 23.3 min and the FTGC run time was 2.5 min, a time saving of over 20 min per run. This benefit comes at the price of decreased resolution. The calculated resolution of peaks C13:0 (tridecanoic acid methyl ester) and C14:0 (myristic acid methyl ester) was 1.8 for the FTGC run, compared with 4.8 for the GC run. If the library of microbial fatty acid compositions could be translated to match data derived from FTGC, microbial identifications could be made faster.

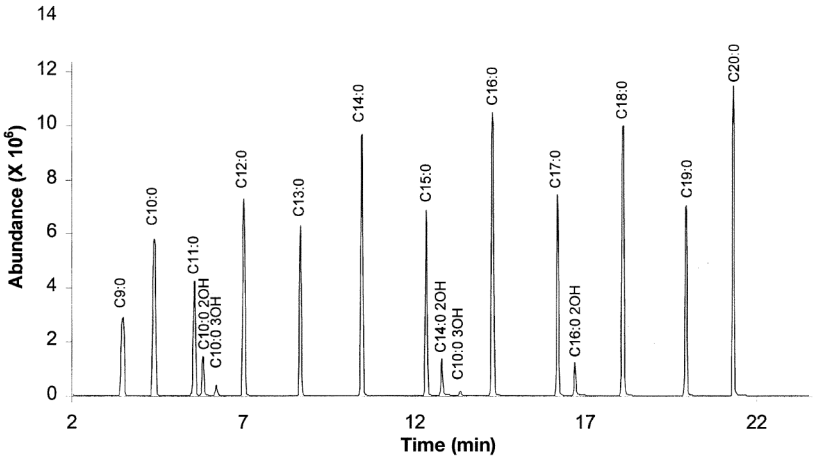

Figure 6. Total ion chromatogram from the GC–MSD analysis of 2 µL of the MIS calibration standards. Chromatographic conditions are listed in Table I, line 7. Codes used to identify the peaks are defined in Table IV.

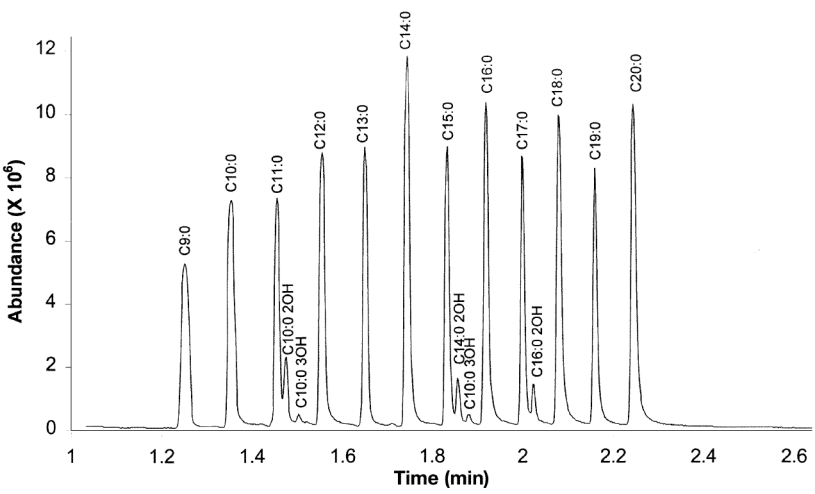

Figure 7. Total ion chromatogram from the FTGC–MSD analysis of 2 µL of the MIS calibration standards. Chromatographic conditions are listed in Table I, line 8. Codes used to identify the peaks are defined in Table IV.

Conclusion

FTGC will save time in any analysis in which chromatographic time is longer than sample preparation time, thereby saving money and increasing throughput. Although peak resolution is decreased, mass spectrometry can be used in conjunction with the FTGC column to quantitate some coeluting compounds. This technique shows promise with analyses such as sugar derivatives and fatty acid esters in which the sample preparation time is short or the samples can be prepared in batches. Analysis of *cis*- and *trans*-fatty acids is problematic because of the loss of resolution and the inability of an MSD to distinguish between the spectra. SPME analysis of food flavors is also not an ideal application for FTGC because the extraction times are usually longer than typical FTGC run times.

Acknowledgments

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Table IV. Names of Esters Corresponding to Codes Used in the Text, Tables, and Figures

Code	Ester	Code	Ester
C4:0	Butyric acid methyl ester	C18:1n9t	Elaidic acid methyl ester
C6:0	Caproic acid methyl ester	C18:1n9c	Oleic acid methyl ester
C8:0	Caprylic acid methyl ester	C18:2n6t	Linolelaidic acid methyl ester
C10:0	Capric acid methyl ester	C18:2n6c	Linoleic acid methyl ester
C11:0	Undecanoic acid methyl ester	C20:0	Arachidic acid methyl ester
C10:0 2OH	2-Hydroxy-decanoic acid methyl ester	C18:3n6	γ-Linolenic acid methyl ester
C10:0 3OH	3-Hydroxy-decanoic acid methyl ester	C20:1	*cis*-11-Eicosenoic acid methyl ester
C12:0	Lauric acid methyl ester	C18:3n3	Linolenic acid methyl ester
C13:0	Tridecanoic acid methyl ester	C21:0	Heneicosanoic acid methyl ester
C14:0	Myristic acid methyl ester	C20:2	*cis*-11,14-Eicosadienoic acid methyl ester
C14:1	Myristoleic acid methyl ester	C22:0	Behenic acid methyl ester
C15:0	Pentadecanoic acid methyl ester	C20:3n6	*cis*-8,11,14-Eicosatrienoic acid methyl ester
C15:1	*cis*-10-Pentadecenoic acid methyl ester	C22:1n9	Erucic acid methyl ester
C14:0 2OH	2-Hydroxy-tetradecanoic acid methyl ester	C20:3n3	*cis*-11,14,17-Eicosatrienoic acid methyl ester
C14:0 3OH	3-Hydroxy-tetradecanoic acid methyl ester	C20:4n6	Arachidonic acid methyl ester
C16:0	Palmitic acid methyl ester	C23:0	Tricosanoic acid methyl ester
C16:1	Palmitoleic acid methyl ester	C22:2	*cis*-13,16-Docosadienoic acid methyl ester
C17:0	Heptadecanoic acid methyl ester	C24:0	Lignoceric acid methyl ester
C17:1	*cis*-10-Heptadecenoic acid methyl ester	C20:5n3	*cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester
C16:0 2OH	2-Hydroxy-hexadecanoic acid methyl ester	C24:1	Nervonic acid methyl ester
C18:0	Stearic acid methyl ester	C22:60	*cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester

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