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Agilent Technologies
Premier Solution Partner

FAME analytics

High throughput with Intuvo 9000

Application Note

Food

ABSTRACT

This application note shows how high throughput analytics can be used for analysis of fatty acid distribution in foods by applying a FAME standard with 37 components with Intuvo 9000 GC by Agilent Technologies. Detection takes place with FID and MSD. As the runtime of this analysis is only approx. 20 min, Intuvo offers significant time savings compared to conventional GC with up to 80 minutes.

INTRODUCTION

Determination of saturated and unsaturated fatty acid composition in foodstuffs is quite widespread in chromatographic analysis. Packaged food products, for example, must have the proportion of saturated fatty acids on their nutrition label whereas there are additional limits for trans-fatty acids in olive oil and baby food [1,2].

It is not just the resulting nutritional values from total fat content that are of interest for consumers. It has been noted for quite some time that unsaturated and poly-unsaturated fatty acids have significant positive effects on consumers' health compared to their saturated counterparts [3,4]. However, recently it became apparent that unsaturated fatty acids in trans-configuration can also hold considerable health risks. The latter occur during industrial hardening of fat, which for example is standard for manufacturing margarine. Consumption of these so-called trans-fatty acids, or rather its glycerol ester, is these days regarded as essential contributor to coronary heart disease, one of the leading cause of death in industrial nations [5-7].

For gas chromatographic analysis, fatty acids are converted through esterification into fatty acid methyl ester (FAME) to provide them with necessary volatility. Reaching a possibly complete base line separation of the components is still a challenge even with modern column technology. While FAME of saturated fatty acids form a homologous line similar to n-alcanes, their unsaturated counterparts contain C=C double bonds, which are present in different numbers as well as in cis- and trans-configuration. This results in a multitude of very similar components with almost identical separation behaviour. Therefore, current GC methods for separation of FAME are based frequently on relatively long capillary columns (60 to 100 m) and running time (60 to 80 min).

Utilizing the Intuvo 9000, runtime can be shortened significantly through extremely high heating rates. The introduced method for FAME analysis shows a separation in less than 20 minutes for the 37 component-mix between C_4 and C_{24}. Combined with the subsequent cooling-down and equilibrium period the run-to-run time is about 27 minutes.

Intuvo 9000 GC presents a novel generation of GC systems, which define gas chromatography in a completely new way through innovative technologies.

Including:

- direct heating oven, shorter cycle duration – planar column design
- fast, reliable column change – click-and-run connections
- no cutting of columns – Intuvo Guard Chip technology
- immediately available system information – intuitive touchscreen
- more space in the lab – compared to conventional benchtop GCs only half the floor space
- simple handling – interactive, proactive maintenance functions

EXPERIMENTAL

Chemicals and solutions

- Supelco 37 components FAME mix

Instrument

- Intuvo 9000 FID
- Agilent 5977A MSD
- ALS (7693)

Software

- OpenLab CDS 2.1

Method parameter

Parameter	
Agilent Intuvo 9000	
Column	HP-88
Column flow (const.)	1.5 ml/min
Carrier gas	helium (MSD)/ hydrogen (FID)
Inlet	split, 250 °C
Detektor FID	260 ºC
Intuvo Guard Chip	follows oven
Intuvo Bus Temp.	350 ºC
ALS	
Syringe	10 µl
Inj. Vol.	1 µl
MSD 5977A	
Mode	SIM (Scan)
Mass range	50 – 400 amu
Ionization	EI, 70eV
Source Temp.	230 ºC
Quad Temp.	150 ºC

RESULTS AND DISCUSSION

Separation of the standard with 37 FAME-components from C4:00 to C22:6 n3 was optimized with an Intuvo 9000-FID system. To identify the sequence of the components conclusively, the analysis was repeated with a coupling of Intuvo 9000-MSD. After optimizing the method parameters, the performed analysis (see Fig. 1) shows an almost complete separation of the 37 components, only the components C20:4 n6 and C22:1 are hardly separated. Tab. 1 features the chromatographic variables of the FAME-mix injection.

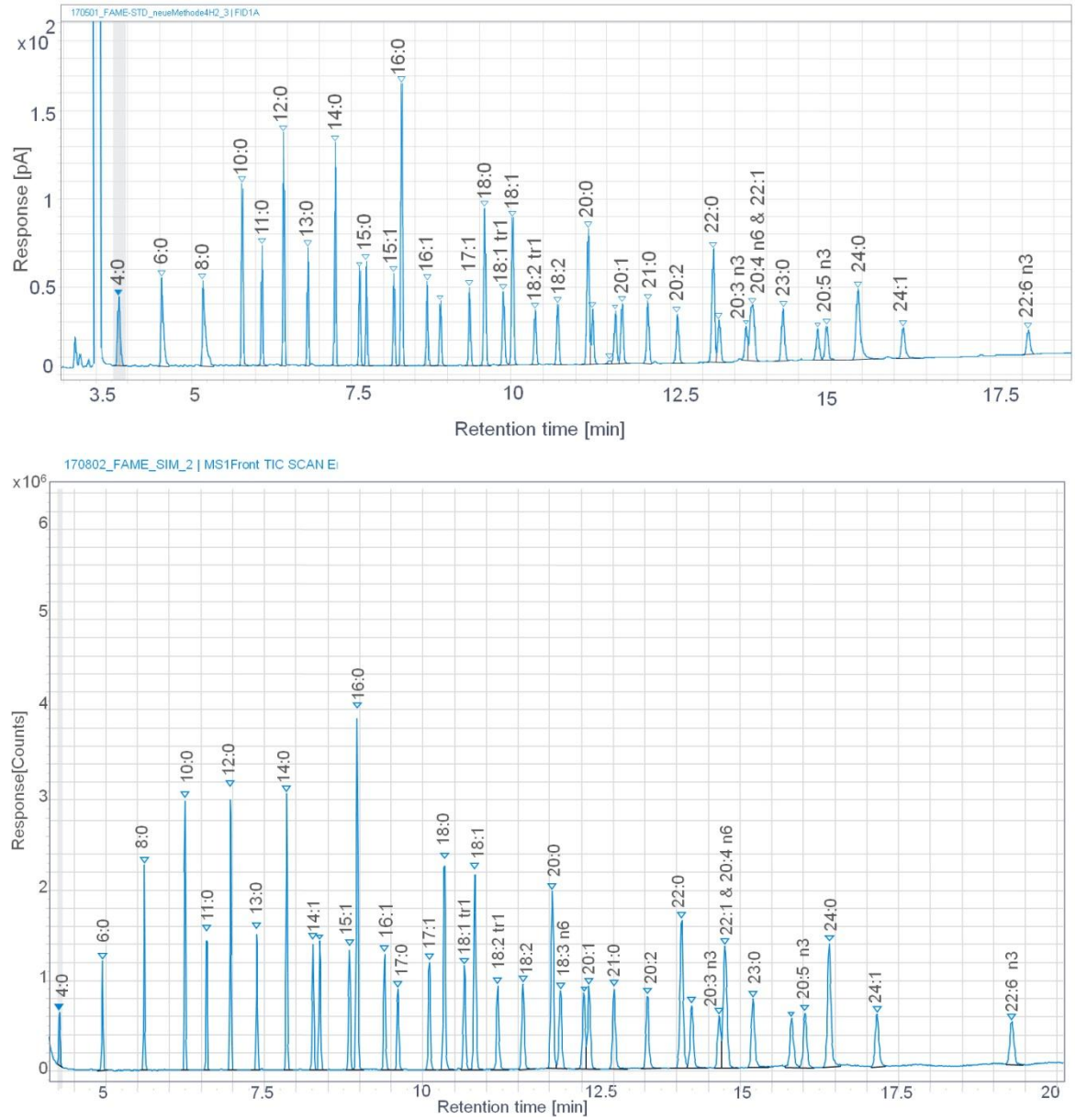

Fig. 1 Complete chromatogram FAME-mix (37 components), tested with INTUVO-FID and carrier gas hydrogen (upper figure), -with INTUVO/MS and carrier gas helium (lower figure) (for complete sequence of elution see Tab. 1)

In the following, practicality of this time-saving method was tested with real samples. The derivatized extracts of 11 different foodstuffs were tested and compared by means of ~~its~~ their fatty acid profiles. As these profiles usually are less complex than the multi-component standard shown in Fig. 1, the mentioned co-elution was of no relevance for the real samples. So, the contents and proportions of saturated, unsaturated, cis- as well as trans-fatty acids could be determined without any problem.

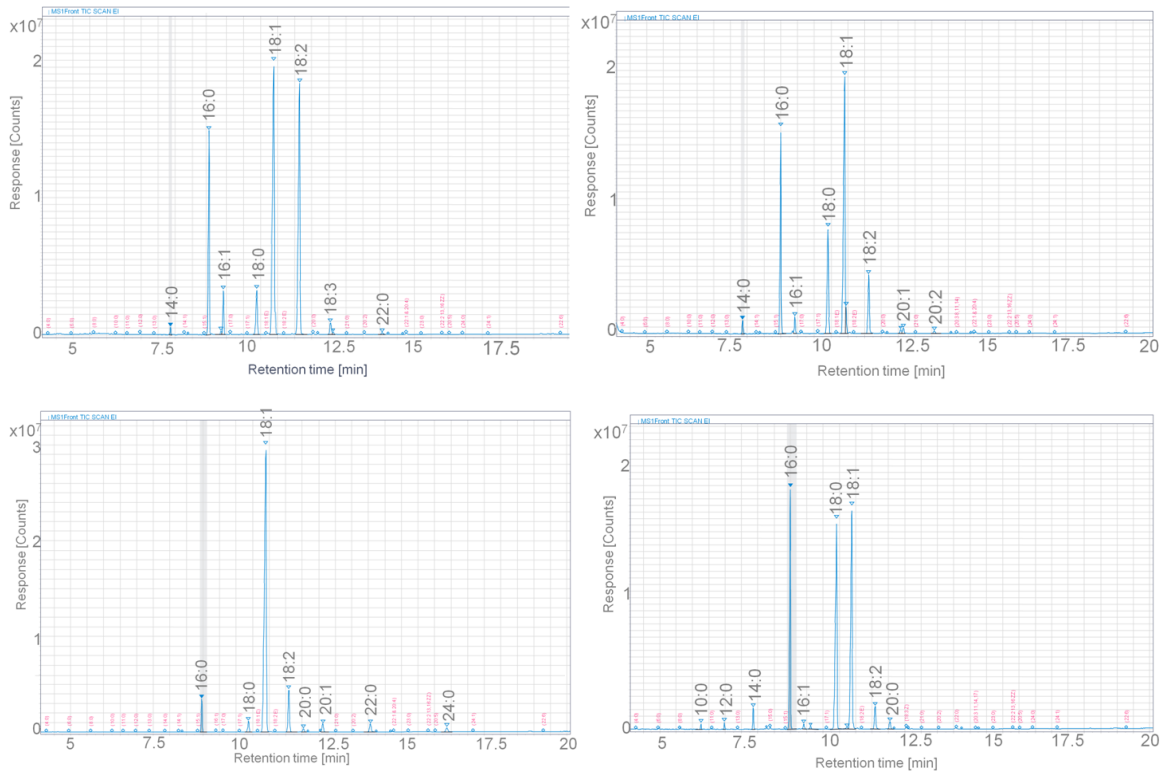

Fig. 2 Exemplary chromatograms of four real samples: meat sausage and pork neck (upper figures from left to right); peanut flips and chocolate (lower figures from left to right)
(tested with INTUVO/MS, components below detection limits are marked in red)

As FAMEs are generally determined by GC-FID, this instrument was used to ensure reproducibility of the results shown. Overall, there has been a high reproducibility with relative standard deviation (RSD) of < 0.2% regarding retention time (RT) and of < 2.5% regarding peak areas.

Tab. 1 Chromatographic data for FAME-mix injection with INTUVO/MS

#	Fatty acids	RT (min)	Area	Area%
1	4:0	4.269	1018932.342	0.858
2	6:0	4.946	1991640.645	1.676
3	8:0	5.609	2996141.128	2.522
4	10:0	6.249	3799042.074	3.197
5	11:0	6.591	2066131.47	1.739
6	12:0	6.966	4487632.695	3.777
7	13:0	7.383	2392423.52	2.014
8	14:0	7.853	5042313.914	4.244
9	14:1	8.266	2565526.169	2.159
10	15:0	8.373	2558619.274	2.153
11	15:1	8.842	2634688.511	2.217
12	16:0	8.961	7948652.64	6.69
13	16:1	9.396	2757892.316	2.321
14	17:0	9.607	1920274.724	1.616
15	17:1	10.102	2787678.599	2.346
16	18:0	10.34	5578736.514	4.695
17	18:1 tr1	10.655	2877548.956	2.422
18	18:1	10.82	5666175.407	4.769
19	18:2 tr1	11.182	2480284.544	2.088
20	18:2	11.58	2719853.342	2.289
21	20:0	12.041	5671172.731	4.773
22	18:3n6	12.169	2592013.115	2.182
23	18:3n3	12.539	2592697.639	2.182
24	20:1	12.619	2856161.775	2.404
25	21:0	13.014	2866595.746	2.413
26	20:2	13.541	2747904.47	2.313
27	22:0	14.081	5700842.558	4.798
28	20:3 n6	14.238	2617588.308	2.203
29	20:3 n3	14.67	1954098.989	1.645
30	20:4 n6 und 22:1	14.763	5478060.116	4.611
31	23:0	15.205	2788398.639	2.347
32	22:2	15.813	2222840.338	1.871
33	20:5 n3	16.026	2327034.571	1.959
34	24:0	16.409	5480406.629	4.613
35	24:1	17.16	2444573.142	2.057
36	22:6 n3	19.287	2185209.058	1.839

CONCLUSION

The performed analysis shows the separation of the 37 FAME-mix components by Intuvo 9000. The runtime can be shortened from up to 80 minutes to less than 20 minutes with nearly equal separation efficiency.

Reproducibility studies via GC-FID showed that RSD(RT) < 0.5% and RSD(Area) < 2.5% can be achieved. Depending on practical requirement, this method therefore offers significant savings on analysis time.

Typically required sample preparation (extraction, drying, derivatisation) can be automated through utilisation of a CTC PAL autosampler in direct coupling with the GC. Depending on the sample matrix, there are already a number of solutions to various extent available in this context. Through a rational adjusted combination of available preparative and chromatographic options, there can be significant gains in efficiency and precision for FAME analytics.

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