Improved *Cis/Trans* Fatty Acid Analysis of Edible Oils and Fats Using Comprehensive GC×GC–FID

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Introduction

The determination of the fatty acid composition of edible oils and fats (as their methyl esters or FAMEs) is routinely performed in numerous laboratories all over the world. The level of detail that is required depends on the intended use of the data. For label-claim purposes, a distinction between saturated, mono-unsaturated, and poly-unsaturated fatty acids generally suffices. Studies on the health impact of fat consumption, on the other hand, require detailed information on chain length distributions, number and positions of double bonds, as well as double-bond orientations (*cis vs. trans*).

The standard method for *trans*-FAME analysis is capillary GC on a 50 to 100 meter highly polar cyanopropyl column (*e.g.* CP-Sil 88). With this technique *trans* analysis is possible in relatively "simple" samples, such as untreated or mildly processed vegetable oils and fish oils. For more complex samples, more sophisticated methods, such as combined HPLC with silver-ion columns (Ag^+-HPLC), and GC are needed. Such systems provide a good separation, but only at the expense of an increased complexity and longer analysis time.

In this contribution we will demonstrate the powerful characteristics of comprehensive GC×GC with FID detection for *trans* fatty acid analysis of commercial fat blends and processed oil samples. The comprehensive GC×GC system applied combines two widely used columns in FAME analysis: the 100% methyl-silicone column in the first dimension and the cyanopropyl column in the second.

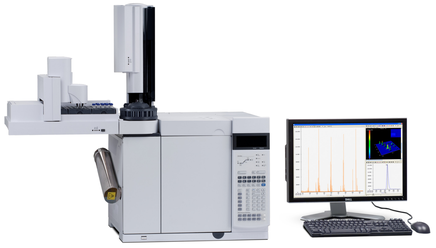
Sample Preparation

For sample preparation, 100 mg of edible oil or fat is weighed into a 10 ml vial. The sample is diluted in 5 ml methyl *tert*-butyl ether (MTBE), and mixed for 60 seconds with a vortex mixer. A 500 µl aliquot is transferred to a 2 ml autosampler vial, and 250 µl trimethylsulfoniumhydroxide (TMSH) derivatisation solution (Macherey-Nagel, Düren, Germany), is added and mixed during 60 seconds with a vortex mixer. 1 µl of the derivatised sample is injected in the GC×GC-FID system.

- Weigh 100 mg edible oil or fat in a 10-ml vial
- Add 5 ml of MTBE
- Mix for 60 seconds with a vortex mixer
- Transfer a 500-µl aliquot to a 2-ml autosampler vial
- Add 250 µl TMSH solution
- Mix for 60 seconds using a vortex mixer
- Inject 1 µl into the GC×GC-FID system

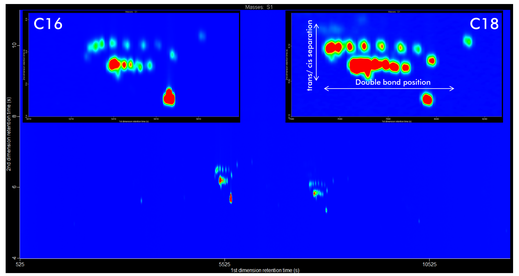
System Parameters

Instrument	LECO GC×GC-FID
1st Dimension Column	Rtx-1, 30 m x 250 µm x 0.1 µm (Restek)
2nd Dimension Column	VF-23ms, 2 m x 100 µm x 0.1 µm (Agilent)
Injector	Split/splitless, split 100:1, 250°C
Carrier Gas	Helium @ 0.5 ml/min constant flow
1st Dimension Oven	120°C (1 min) – 0.4°/min – 215°C (1 min)
2nd Dimension Oven	+10°C relative to 1st dimension oven
Modulator	+20°C relative to 2nd dimension oven
Modulation Period	15 seconds, 1.5 second hot pulse
Detector	FID, 250°C H_2 flow: 40 ml/min Air flow: 450 ml/min N_2 Makeup flow: 45 ml/min

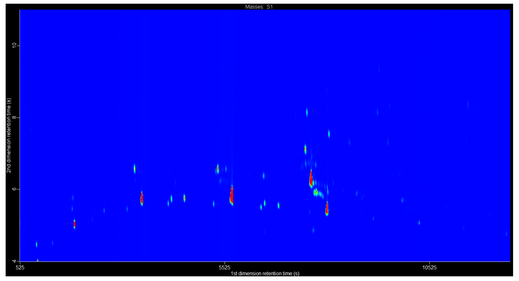


GCxGC-FID

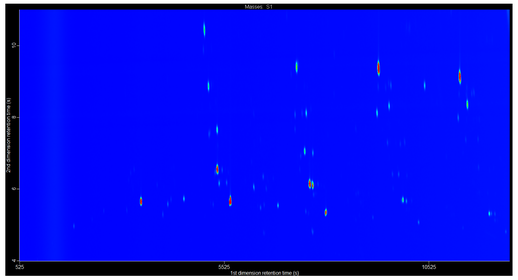
GC×GC–FID



C16:0, C16:1, C18:0, C18:1 including double bond isomers.

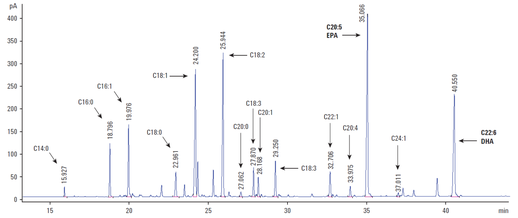


Real butter sample.



Salmon oil sample.

GC–FID



GC–FID chromatogram of a salmon oil sample. Not all peaks could get identified and cis/trans quantification is not possible.

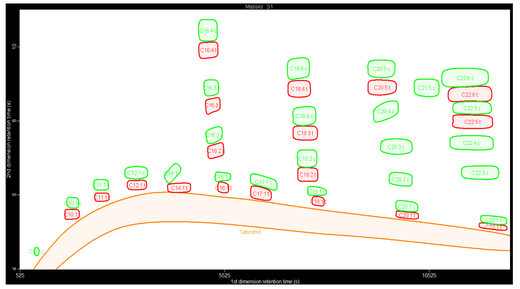
Percent distribution of fatty acids in salmon oil sample.

C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
0.71	4.68	7.95	3.54	12.95	13.86	5.94

C20:0	C20:1	C20:4	C20:5	C22:1	C22:6	C24:1
0.36	1.71	0.87	23.79	3.35	19.93	0.36

Classification

One of the basic features of the LECO ChromaTOF® software of data acquisition and processing is classification. This feature makes it possible to assign regions of the contour plot to classes of compounds. Based on the contour plots of real butter and fish oil, the template below was generated.



Classifications template.

Quantification

Fish oil is well known as an excellent source of polyunsaturated omega-3 fatty acids from which eicosapentaenoic acid (EPA, C20:5 c), and docosahexaenoic acid (DHA, C22:6 c) are the most important. These fatty acids help to reduce the level of cholesterol, and are good for the heart and blood vessels. After automatic projection of the classification template over the contour plot of the fish oil blend by the ChromaTOF software, both the percent distribution of total saturated, cis, and trans fatty acids, as well as the percentage contribution of each individual sub-class can be automatically filtered from the resulting peak table.

Percent distribution of Saturated, Cis, and Trans content of salmon oil.

Saturated	Cis	Trans
30.13	67.68	2.19

Percent distribution of classes and individuals.

Fatty Acid	%	Fatty Acid	%	Fatty Acid	%
C10:0	0.14	C17:1 t	0.49	C20:4 c	1.65
C12:0	7.58	C18:0	3.55	EPA	17.67
C14:0	0.80	C18:1 c	11.83	C20:5 t	0.26
C16:0	17.00	C18:1 t	0.12	C21:5 c	0.72
C16:1 c	8.26	C18:2 c	1.67	C22:0	0.10
C16:1 t	0.63	C18:3 c	1.11	C22:1 c	0.84
C16:2 c	1.43	C18:4 c	2.87	C22:4 c	0.09
C16:3 c	1.45	C19:0	0.08	C22:5 c	2.13
C16:4 c	2.17	C20:0	0.20	C22:5 t	0.31
C16:4 t	0.12	C20:1 c	1.35	DHA	11.69
C17:0	0.67	C20:2 c	0.26	C22:6 t	0.25
C17:1 c	0.19	C20:3 c	0.31		

Conclusion

The determination of FAME composition in edible oils and fats is classically performed by GC–FID. However, it is proven here that this technique is not able to give a full quantitative finger print of the fatty acid contribution.

GC×GC–FID makes it possible to perform full separation of saturated and (poly)unsaturated fatty acids, including the separation on their double bond position plus *cis/trans* orientation. This makes GC×GC–FID a very powerful system in the daily routine for label-claim purpose.