Application Note

Clinical Research

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Fatty Acid Analysis in Biological Samples by GC/FID/MS Using the Agilent Intuvo 9000 GC

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Abstract

The fatty acid composition of the lipid fraction in biological samples has become an important marker in clinical research. In this application note, two methods are described for the analysis of fatty acids as methyl esters (FAMEs) using the Agilent Intuvo 9000 GC. One method is based on a GC/MS configuration with backflush option; the second method includes parallel MS and FID detection. Using a long polar Agilent J&W Select FAME Intuvo GC column, all target FAMEs, including C18:1 *cis-trans* and positional isomers, conjugated linoleic acids (CLAs), and polyunsaturated omega-3 and omega-6 essential and metabolic fatty acids (EMFAs) were separated. Excellent retention time stability and peak area repeatability were obtained, while the method was found robust for routine applications in a clinical research laboratory environment thanks to the implementation of backflush or Agilent Guard Chip temperature programming.

Introduction

The analysis of FAMEs is a very important GC application. This analysis is typically performed on a GC/FID instrument, and, depending on sample complexity and analytical demand, short or very long capillary columns coated with polar polyethylene glycol (WAX) or cyanopropyl (DB-23, HP-88, CPSil-88) stationary phases are used. From these analyses, the fatty acid composition is obtained, including saturated (SFA), mono-unsaturated (MUFA), polyunsaturated (PUFA), and *trans*-fatty acids.

In recent years, the interest in FAME analysis in clinical research has grown substantially. With these analyses, the levels of EMFAs are measured in whole blood, plasma, or red blood cells (RBC). A detailed profile of C12 to C26 fatty acids provides information regarding mitochondrial and peroxisomal fatty acid metabolism. Based on essential and metabolic fatty acids (EMFA) analysis, the need for fatty acid supplementation and/or dietary modification can be defined. Levels of specific fatty acids have also been used to diagnose cystic fibrosis,[1] and it is expected that this analysis will become more important in the future.

In detailed fatty acid profiling of biological matrices such as blood, plasma, or red blood cells, a range of specific fatty acids are monitored, including omega-3 fatty acids, omega-6 fatty acids, omega-9 fatty acids, saturated fatty acids, monounsaturated fats, *trans* fats, and conjugated linoleic acids (CLAs). Concentration levels range over three orders of magnitude with highly abundant fatty acids at µmol/mL levels (in plasma), while other EMFAs are present at nmol/mL level. In food products, FAME analysis is typically done using GC/FID, since the focus is on the most abundant fatty acids. For clinical research, on the other hand, there is a clear trend towards GC/MS for EMFA analysis because of the low levels of some target compounds.[2] Although MS provides additional identification, selectivity, and sensitivity, the chromatographic separation remains crucial, since separation of positional isomers (location of double bonds) and geometric isomers (*cis-trans*) is mandatory, and MS detection does not allow unequivocal differentiation of isomers based on electron ionization mass spectra. GC separation of these isomers requires long polar cyanopropyl columns and delicate flow and temperature program optimization. Therefore, a relatively long analysis time is incurred.

A specific challenge in FAME analysis in clinical research is the presence of some high-molecular-weight (HMW) compounds, such as cholesterol, that are co-extracted with the lipid fraction from biological samples. Since only small sample amounts are typically used, and methods should be applicable in a routine environment, additional sample preparation steps such as solid phase extraction are not frequently used. Therefore, long bake-out times at the highest possible temperature are often applied. The maximum operating temperature of highly polar cyanopropyl columns is limited, and bake-out at maximum temperature results in faster column degradation. The robustness of these columns is therefore questioned.

In this application note, two GC configurations based on the Intuvo 9000 GC are presented. In configuration 1, a 50 m J&W Select FAME Intuvo GC column was used for separation and a postcolumn backflush Flow Chip was installed, allowing removal of HMW material during postrun. In the second configuration, the same column was used with column effluent splitting to MS and FID. The latter detector allows area % reporting, as is often used in FAME analysis, while MS can be used for peak identification and trace analysis, eventually applying selection ion monitoring (SIM) mode. Since no column backflush was applied here, the inertness of the analytical system was maintained by using temperature programming of the Guard Chip.

Both configurations were tested using standard mixtures and extracts from red blood cells. Separation, stability of retention times, repeatability of peak areas, and method robustness were verified. The fast oven cooling, easy method start-up, and diagnostic tools incorporated in the Intuvo 9000 GC are additional benefits, making these configurations attractive for routine operation in a clinical research laboratory environment.

Experimental

Standard and samples

Two standard mixtures were used. The first mixture was a 37-component stock standard (Merck-Sigma, cat. number CRM47885), containing saturated and unsaturated fatty acids from C4 to C24. The stock standard (varied concentrations 200 to 600 µg/mL each solute) was diluted in 20 mL of toluene.

In addition, a more complex standard mixture was prepared by mixing 200 µL of the diluted 37-component mixture with CLA mix (Merck-Sigma cat. number O 5632), C18:2 mix (tt, tc, ct, cc) (Merck-Supelco, cat. number 47791), C18:3 mix (Merck-Supelco, cat. number 47792), C18:1 c11, C18:1 t6, C18:1 t11, and C18:1 c6 (from pure solutes, Merck-Supelco). The concentration of all solutes was adjusted to 10 to 30 µg/mL.

Red blood cells (RBC) were used as biological sample. This RBC fraction was obtained from whole blood. One milliliter was diluted with physiological water (4 mL), centrifuged (5 minutes, 3000 rpm), and the upper layer was removed. The remaining fraction in the bottom of the centrifuge tube was used as the RBC fraction.

Note: Several nomenclatures are used to specify fatty acids. Target compounds and detected solutes in the RBC sample are listed in Table 1, together with the end carbon (n) or omega annotation (column 1), delta annotation (column 2), IUPAC name of the corresponding acid (column 3), common name of the acid (column 4) and fatty acid class (column 5). The solutes present in the 37-component mixture are marked in column 6. The n or omega annotation given in column 1 is used for peak labeling in the chromatograms.

Table 1. List of target FAMES. FA Classes: SFA: saturated fatty acids, MUFA: mono-unsaturated fatty acids, t-MUFA: trans mono-unsaturated fatty acid, PUFA: poly-unsaturated fatty acid, t-PUFA: trans poly-unsaturated fatty acid, w3-PUFA: omega 3 poly-unsaturated fatty acid, CLA: conjugated linoleic acid.

Annotation	Analyte Code				37K	RT (min)	RT (min)		EI (70 eV)
(n or ω)	(delta)	IUPAC Name Acid	Common Name Acid	FA Class	Mix	Config. 1	Config. 2	MM	Base Peak
C12:0	C12:0	Dodecanoic	Lauric	SFA	x	9,219	9,223	214	74
C13:0	C13:0	Tridecanoic		SFA	x	10,172	10,101	228	74
C14:0	C14:0	Tetradecanoic	Myristic	SFA	x	11,396	11,247	242	74
C14:1 n5	C14:1 c-9	*cis*-9 Tetradecenoic	Myristoleic	MUFA	x	12,297	12,099	240	55
C15:0	C15:0	Pentadecanoic		SFA	x	12,967	12,716	256	74
C15:1	C15:1	*cis*-9 Pentadecenoic		MUFA	x	14,134	13,817	254	55
C16:0	C16:0	Hexadecanoic	Palmitic	SFA	x	14,994	14,619	270	74
C16:1 n7	C16:1 c-9	*cis*-9 Hexadecenoic	Palmitoleic	MUFA	x	16,121	15,699	268	55
C17:0	C17:0	Heptadecanoic		SFA	x	17,460	16,967	284	74
C17:1 n8	C17:1 c-9	*cis*-9 Heptadecenoic		MUFA	x	18,847	18,309	282	55
C18:0	C18:0	Octadecanoic	Stearic	SFA	x	20,498	19,877	298	74
C18:1 n12-t	C18:1 t-6	*trans*-6 Octadecenoic	*trans*-Petroselenic	t-MUFA		21,243	20,605	296	55
C18:1 n9-t	C18:1 t-9	*trans*-9 Octadecenoic	Elaidic	t-MUFA	x	21,347	20,700	296	55
C18:1 n7-t	C18:1 t-11	*trans*-11 Octadecenoic	*trans*-Vaccenic	t-MUFA		21,567	20,904	296	55
C18:1 n12	C18:1 c-6	*cis*-6 Octadecenoic	(*cis*-) Petroselenic	MUFA		21,705	21,041	296	55
C18:1 n9	C18:1 c-9	*cis*-9 Octadecenoic	Oleic	MUFA	x	21,844	21,177	296	55
C18:1 n7	C18:1 c-11	*cis*-11 Octadecenoic	(*cis*-) Vaccenic	MUFA		22,110	21,449	296	55
C18:2 n6-tt	C18:2 t-9,t-12	*trans*-9, *trans*-12 Octadecadienoic		t-PUFA	x	23,115	22,409	294	67
C18:2 n6-ct	C18:2 c-9,t-12	*cis*-9, *trans*-12 Octadecadienoic		t-PUFA		23,727	23,017	294	67
C18:2 n6-tc	C18:2 t-9,c-12	*trans*-9, *cis*-12 Octadecadienoic		t-PUFA		24,027	23,305	294	67
C18:2 n6	C18:2 c-9,c-12	*cis*-9, *cis*-12 Octadecadienoic	Linoleic	PUFA	x	24,281	23,557	294	67
C18:3 n3-ttt	C18:3 ttt	*trans*-9,12,15 Octadecatrienoic		t-PUFA		25,309	24,553	294	67
C18:3 n6	C18:3 c-12	*cis*-6,9,12 Octadecatrienoic	γ-Linolenic (GLA)	PUFA	x	26,025	25,255	292	79
C18:3 n3 ttc+tct	C18:3 ttc+tct	*trans*-9, *trans*-12, *cis*-15 Octadecatrienoic + *trans*-9, *cis*-12, *trans*-15 octadecatrienoic		t-PUFA		26,187	25,418	292	79
C18:3 n3 ctt+cct	C18:3 ctt+cct	*cis*-9, *trans*-12, *trans*-15 Octadecatrienoic + cis-9, *cis*-12, *trans*-15 octadecatrienoic		t-PUFA		26,430	25,648	292	79

Annotation	Analyte Code	IUPAC Name Acid	Common Name Acid	FA Class	37K Mix	RT (min)	RT (min)	MM	EI (70 eV)
(n or ω)	(delta)					Config. 1	Config. 2		Base Peak
C18:3 n3 ctc	C18:3 c-9,t-12,c-15	*cis*-9, *trans*-12, *cis*-15 Octadecatrienoic		t-PUFA		27,019	26,220	292	79
C18:3 n3 tcc	C18:3 t-9,c-12,c-15	*trans*-9, *cis*-12, *cis*-15 Octadecatrienoic		t-PUFA		27,175	26,382	292	79
C18:3 n3	C18:3 c-9,12,15	*cis*-9,12,15 Octadecatrienoic	α-Linolenic (ALA)	ω3-PUFA	x	27,435	26,634	292	79
C20:0	C20:0	Eicosanoic	Arachidic	SFA	x	27,862	27,027	326	74
C18:2-CLA 1+2	C18:2 c-9, t-11 + C18:2 t-9, c-11	*cis*-9, *trans*-11 + *trans*-9, *cis*-11-Octadecadienoic	Rumenic (c9,t11) (=CLA1) + CLA 2	CLA		28,434	27,609	294	67
C18:2-CLA 3	C18:2 t-10, c-12	*trans*-10,*cis*-12 Octadecadienoic	CLA3	CLA		29,023	28,180	294	67
C20:1 n9	C20:1 c-11	*cis*-11 Eicosenoic	EA	MUFA	x	29,491	28,631	324	55
C21:0	C21:0	Heneicosanoic		SFA	x	32,055	31,142	340	74
C20:2 n6	C20:2 c-11,14	*cis*-11,14 Eicosadienoic	EDA	PUFA	x	32,534	31,619	322	67
C20:3 n6	C20:3 c-8,11,14	*cis*-8,11,14 Eicosatrienoic	Dihomo-γ-linolenic, DGLA	PUFA	x	34,532	33,600	320	79
C20:4 n6	C20:4 c-5,8,11,14	*cis*-5,8,11,14 Eicosatetranoic	Arachidonic, AA	PUFA	x	35,959	35,000	318	79
C20:3 n3	C20:3 c-11,14,17	*cis*-11,14,17 Eicosatrienoic		ω3-PUFA	x	36,225	35,267	320	79
C22:0	C22:0	Docosanoic	Behenic	SFA	x	36,519	35,535	354	74
C22:1 n9	C22:1 c-13	*cis*-13 Docosenoic	Erucic	MUFA	x	38,385	37,390	352	55
C20:5 n3	C20:5 c-5,8,11,14,17	*cis*-5,8,11,14,17 Eicosapentaenoic	EPA	ω3-PUFA	x	39,852	38,858	316	79
C23:0	C23:0	Tricosanoic		SFA	x	41,041	40,048	368	74
C22:2 n6	C22:2 c-13,16	*cis*-13,16 Docosadienoic		PUFA	x	41,677	40,735	350	81
C24:0	C24:0	Tetracosanoic	Lignoceric	SFA	x	44,847	43,922	382	74
C22:4 n6	C22:4 c-7,10,13,16	*cis*-7,10,13,16 Docosatetraenoic		PUFA		45,032	44,100	346	79
C24:1 n9	C24:1 c-15	*cis*-15 Tetradecenoic acid	Nervonic	MUFA	x	46,251	45,243	380	55
C22:5 n3	C22:5 c-7,10,13,16,19	*cis*-7,10,13,16,19 Docosapentaenoic	DPA	ω3-PUFA		47,770	46,622	344	79
C22:6 n3	C22:6 c-4,7,10,13,16,19	*cis*-4,7,10,13,16,19 Docosahexaenoic	DHA	ω3-PUFA	x	48,613	47,377	342	79
C16:0-DMA		C16:0-Dimethyl acetal	Palmitaldehyde dimethyl acetal			13,643		286	75
C18:0-DMA		C18:0-Dimethyl acetal	Stearaldehyde dimethyl acetal			18,414		314	75
C18:1-DMA		C18:1-Dimethyl acetal				19,459		312	75

Sample preparation

FAMEs are prepared from the RBC fraction using a slightly modified Lepage and Roy method:[3]

1. 200 µL red blood cell fraction was transferred in a 10 mL headspace vial.
2. 2 mL of a methanol/toluene (80/20) mixture was added, and the solution was vortexed for 30 seconds.
3. 200 µL acetyl chloride was slowly added (*).
4. The vial was capped and thermostatted at 100 °C for one hour.
5. The sample was cooled at 4 °C for 10 minutes.
6. 5 mL of cold (4 °C) 6% K_2CO_3 in water was added.
7. The sample was vortexed (30 seconds).
8. 1 mL of hexane was added.
9. The sample was vortexed and centrifuged.
10. The upper layer was transferred to a 1.8 mL high recovery vial.
11. The extract was concentrated to approximately 100 µL under nitrogen.

Note: (*) The reaction with acetyl chloride is very exothermic and can cause splashing. It should be performed in a fume hood with great care, using protection (safety glasses, gloves, etc.).

GC parameters

GC/FID analyses were performed on an Agilent Intuvo 9000 Series gas chromatograph (GC). The system was equipped with a split/splitless inlet, a pneumatic switching device (PSD), a flame ionization detector (FID), an Agilent 5977B GC/MSD with Inert Plus EI source, and an Agilent 7693A automatic liquid sampler (ALS) with 10 µL syringe.

For configuration 1, an Agilent Intuvo D2-MS postcolumn backflush chip (p/n G4588-60322) was installed. The GC/MS parameters used with this configuration are summarized in Table 2.

Backflush is applied to remove co-extracted, late-eluting compounds such as cholesterol. If no backflushing were applied, these compounds would elute after several runs as ghost peaks and/or contaminate the column and MS source. Bake-out at high temperatures is not a good option for highly polar cyanopropyl columns with limited maximum operating temperature.

Table 2. GC/MS parameters for FAME analysis using configuration 1.

Parameter	Value
Inlet	Splitless: 250 °C, splitless liner UI (p/n 5190-2293)
Injection Volume	1 µL
Column	Agilent J&W Select FAME GC column module, 50 m × 0.25 mm, 0.25 µm (p/n CP7419-INT)
Column Pressure	250 kPa helium, constant pressure (5 kPa during postrun)
PSD	50 kPa constant pressure (200 kPa during postrun)
Column Temperature Program	70 °C (3 minutes), 40 °C/min to 150 °C (5 minutes), 1 °C/min to 180 °C, 3 °C/min to 210 °C (= 50 minutes) Postrun: 15 minutes at 210 °C
Intuvo Guard Chip Temperature	Track oven mode
Intuvo Bus Temperature	250 °C
Intuvo Detector Tail	300 °C
MSD	EI (70 eV), source: 230 °C, quadrupole: 150 °C Scan mode: 45 to 550 amu Solvent delay: 8 minutes

Backflush was implemented during the postrun. Using a reduced inlet pressure (5 kPa), increased outlet pressure (PSD at 200 kPa), and 210 °C column temperature, the column flow was inverted (–0.41 mL/min). A postrun time of 15 minutes was used (2.5 void times). The backflush configuration and conditions are illustrated in Figure 1.

For configuration 2, an Agilent D1-MS splitter chip 1:1 (p/n G4588-60502) was installed. The GC/MS parameters used on configuration 2 are summarized in Table 3.

With this configuration, a Guard Chip temperature program was used. After injection (and a short initial isothermal hold), the Guard Chip temperature was programmed at 100 °C/min to 150 °C to transfer the injected solutes of interest to the column. After reaching 150 °C, the Guard Chip was programmed down to 50 °C and held at that temperature until the end of the analysis. The maximum temperature of 150 °C was selected based on tests verifying complete transfer of all solutes of interest to the analytical column. Lower temperatures were not sufficient to recover the solute of interest with the highest boiling point (C24:0). At 150 °C Guard Chip temperature, the same peak area was obtained as that for the track oven mode, indicating quantitative transfer.

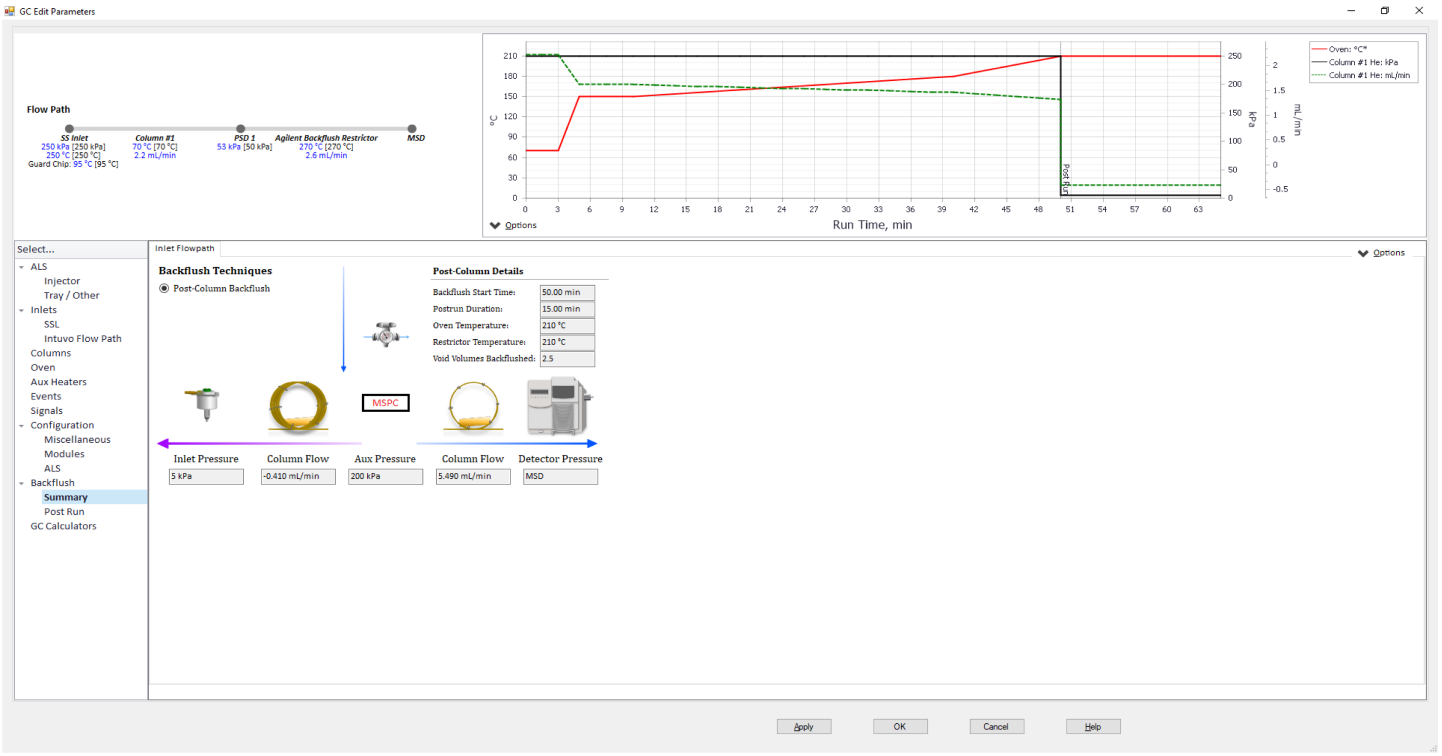

Figure 1. Screenshot of backflush calculator in Agilent MassHunter Acquisition software.

Results and discussion

Configuration 1

Figure 2 shows the total ion chromatogram (TIC) of the GC/MS analysis of the complex FAME mixture. The early eluting FAMEs (C4 to C11) are not shown, since these are typically discarded in FAME analysis in clinical research. A very good separation is obtained, with the last compound of interest (C22:6 n3 = DHA) eluting at 48.6 minutes. An overview of retention times, molecular mass (MM), and base peak in the 70 eV EI mass spectrum are included in Table 1. The most critical separations are the C18:1 and C18:3 isomers. As illustrated by the zoom on the elution window from 20 to 33 minutes in Figure 3, sufficient resolution is obtained to allow differentiation of these isomers. Also, the CLA isomers are well separated and, due to their conjugated double bond, these elute after C18:3 isomers on the highly polar cyanopropyl column. The high column length and optimized column flow and temperature program are mandatory to obtain these separations.

Table 3. GC/MS parameters for FAME analysis using configuration 2.

Parameter	Value
Inlet	Splitless: 250 °C, splitless liner UI (p/n 5190-2293)
Injection Volume	1 µL
Column	Agilent J&W Select FAME GC column module, 50 m × 0.25 mm, 0.25 µm (p/n CP7419-INT)
Column Pressure	250 kPa helium, constant pressure
PSD	10 kPa constant pressure
Column Temperature Program	60 °C (3 minutes), 40 °C/min to 150 °C (5 minutes), 1 °C/min to 180 °C, 4 °C/min to 210 °C (3 minutes) (= 50.75 minutes) Postrun: 15 minutes at 210 °C
Intuvo Guard Chip Temperature	100 °C (3 minutes), 100 °C/min to 150 °C, 100 °C/min to 50 °C
Intuvo Bus Temperature	250 °C
Intuvo Detector Tail	300 °C
FID Detector	Flame ionization at 300 °C 40 mL/min hydrogen, 400 mL/min air
MSD	EI (70 eV), source: 230 °C, quadrupole: 150 °C Scan mode: 45 to 550 amu Solvent delay: 8 minutes

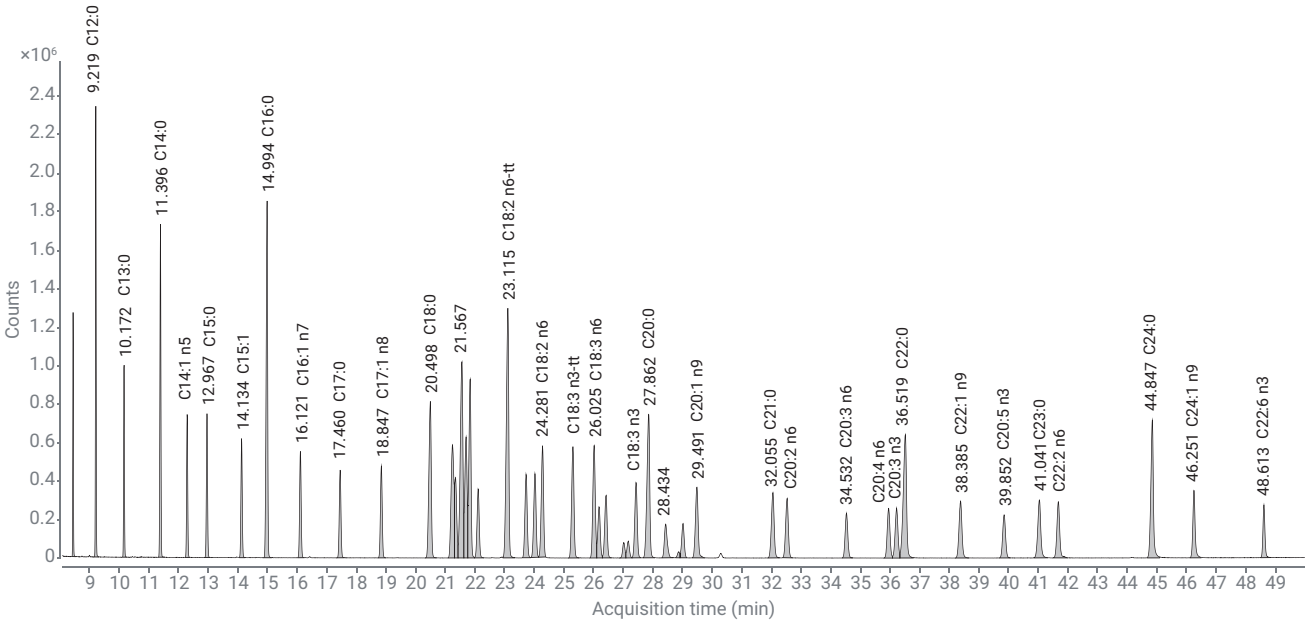

Figure 2. TIC of GC/MS analysis of complex FAME mixture.

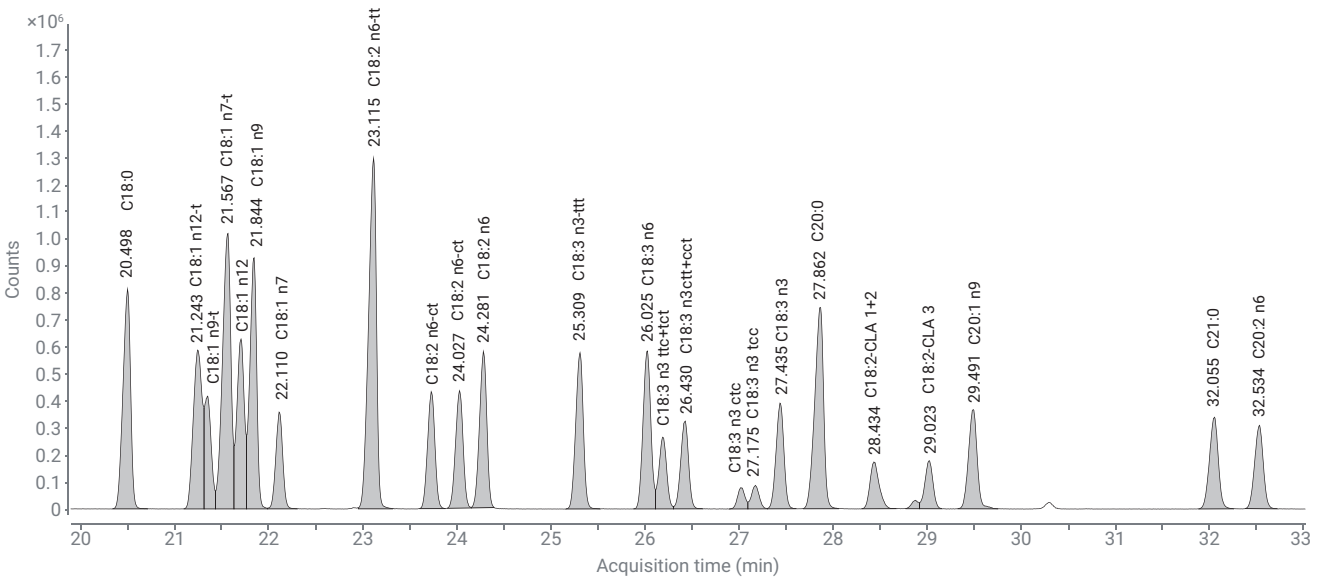

Figure 3. TIC of GC/MS analysis of complex FAME mixture (zoom 20 to 33 minutes).

The TIC obtained for a red blood cell extract is shown in Figure 4. Main fatty acids are C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 n9 (oleic acid), C18:2 n6 (linoleic acid), and C20:4 n6 (arachidonic acid). An interesting feature is the detailed separation of polyunsaturated fatty acids, including C22:4 n6, C22:5 n3 (DPA), and C22:6 (DHA). The RBC extract also contains dimethyl acetals of the respective aldehydes of C16:0, C18:0, and C18:1. This was also reported earlier for FAME analysis in whole blood samples.[4] A zoom on this chromatogram, shown in Figure 5, shows that very detailed information is obtained and that minor fatty acids, such as C18:1 n7-t (*trans*-vaccenic acid), C18:1 n7 (*cis*-vaccenic acid), C20:1 n9 (eicosenoic acid), and C20:5 n3 (EPA), can also be detected.

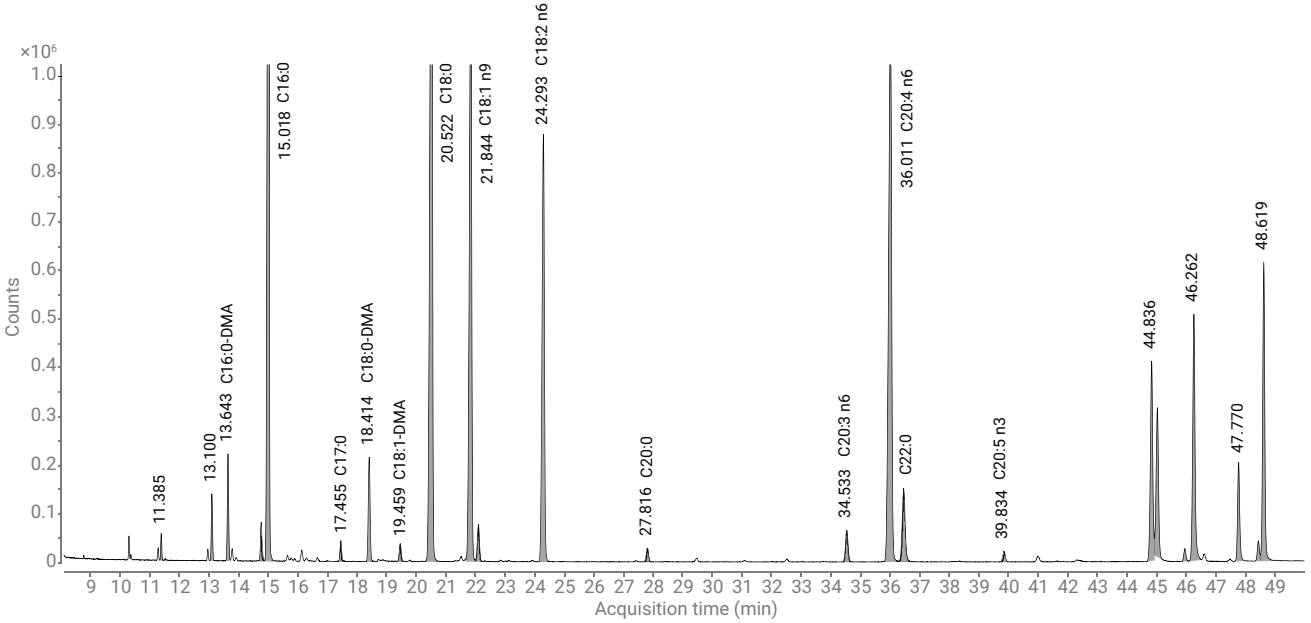

Figure 4. TIC of GC/MS analysis of red blood cell FAMEs using configuration 1.

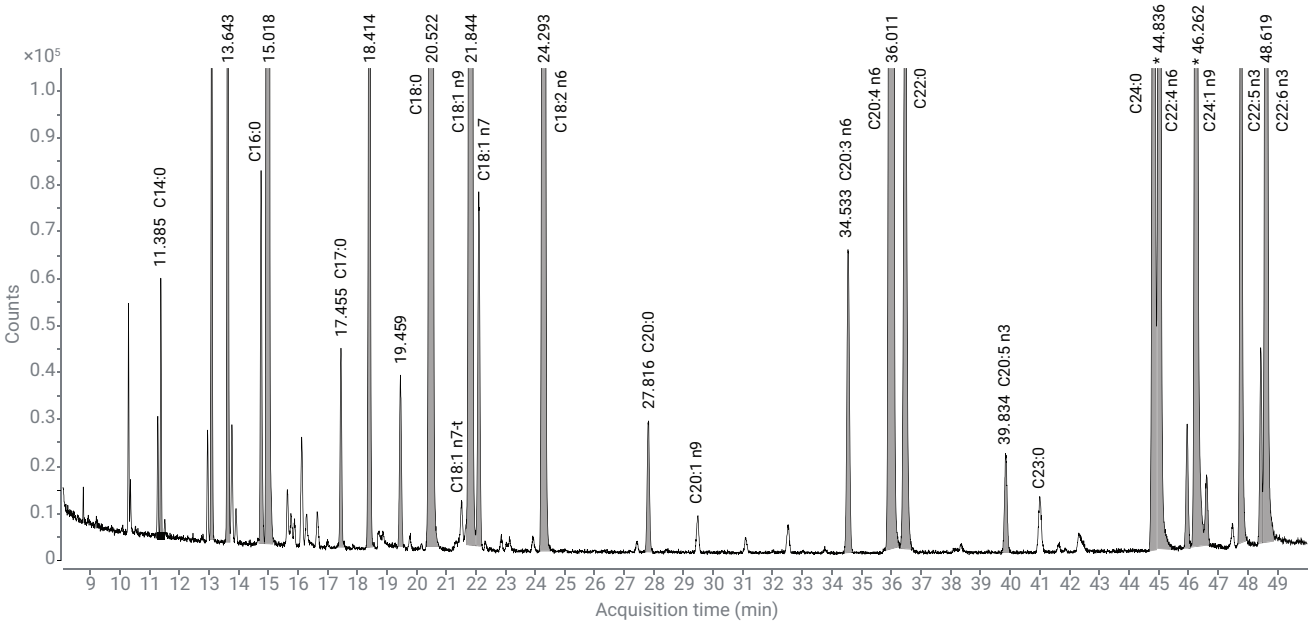

Figure 5. TIC of GC/MS analysis of red blood cell FAMEs using configuration 1 (zoom).

The repeatability of this analysis was tested by analyzing six aliquots of a pooled RBC sample. The average retention time repeatability and peak area repeatability are given in Table 4. Retention time stability for the real samples typically shows a standard deviation of less than one second. The absolute peak areas for the main peaks obtained by integration of the TIC are below 10% RSD, and for most solutes below 5% RSD.

These results clearly demonstrate that excellent separation of all target compounds, including C18:1 isomers, *cis-trans* isomers, conjugated linoleic acids (CLA) and polyunsaturated ω3 and ω6 essential and metabolic fatty acids (EMFAs) are obtained in 50 minutes on this GC configuration. Cholesterol and other HMW compounds are backflushed, keeping the analytical system clean. This results in very good retention time stability on the very polar cyanopropyl column and excellent peak area repeatability is also obtained.

Table 4. Average retention times and peak areas for main fatty acids detected in RBC. Standard deviations and relative standard deviations (RSD%) (n = 6) are also given.

Solute	Retention Time			TIC Peak Area		
	Mean (min)	Std. Dev. (min)	RSD (%)	Mean area	Std. Dev.	RSD%
C14:0	11.385	0.000	0.00	136756	3710	2.71
C15:0	12.958	0.003	0.02	69762	2801	4.02
C16:0-DMA	13.637	0.000	0.00	860847	33086	3.84
C16:0	15,009	0.003	0.02	11703009	217557	1.86
C17:0	17.443	0.000	0.00	182251	9868	5.41
C18:0-DMA	18.405	0.003	0.02	1478627	97392	6.59
C18:1-DMA	19.446	0.005	0.02	294215	22384	7.61
C18:0	20.509	0.002	0.01	10782921	209350	1.94
C18:1 n9	21.827	0.004	0.02	6953331	109935	1.58
C18:1 n7	22.086	0.005	0.02	365041	16796	4.60
C18:2 n6	24.276	0.005	0.02	5025334	97002	1.93
C20:0	27.784	0.009	0.03	181189	11263	6.22
C20:1 n9	29.447	0.011	0.04	55272	5430	9.82
C20:3 n6	34.517	0.010	0.03	453680	19087	4.21
C20:4 n6	35.994	0.007	0.02	8112510	119340	1.47
C22:0	36.438	0.007	0.02	1089687	37112	3.41
C20:5 n3	39.825	0.008	0.02	157994	8208	5.20
C23:0	40.978	0.007	0.02	97544	9321	9.56
C24:0	44.822	0.006	0.01	2479707	72109	2.91
C22:4 n6	45.014	0.007	0.02	1837317	14890	0.81
C24:1 n9	46.252	0.006	0.01	2969071	81231	2.74
C22:5 n3	47.759	0.005	0.01	1131990	23853	2.11
C22:6 n3	48.611	0.005	0.01	3190662	52500	1.65

Configuration 2

Figure 6 shows the TIC and FID trace obtained for the analysis of the complex FAME mixture using configuration 2 with effluent splitting to FID and MS (1:1 split ratio). Due to a different outlet pressure at the column outlet (in the splitter Flow Chip), the GC conditions were slightly modified to obtain a separation that is very similar to the separation shown in Figure 2. Again, very good resolution is obtained, and the profiles in MS and FID are identical, showing that no peak broadening is generated on either detector.

As illustrated by the zoom on the elution window from 20 to 33 minutes in Figure 7, sufficient resolution is obtained to allow differentiation of the C18:1, C18:2, C18:3, and CLA isomers. The retention times for all detected peaks measured in the TIC using configuration 2 are included in Table 1.

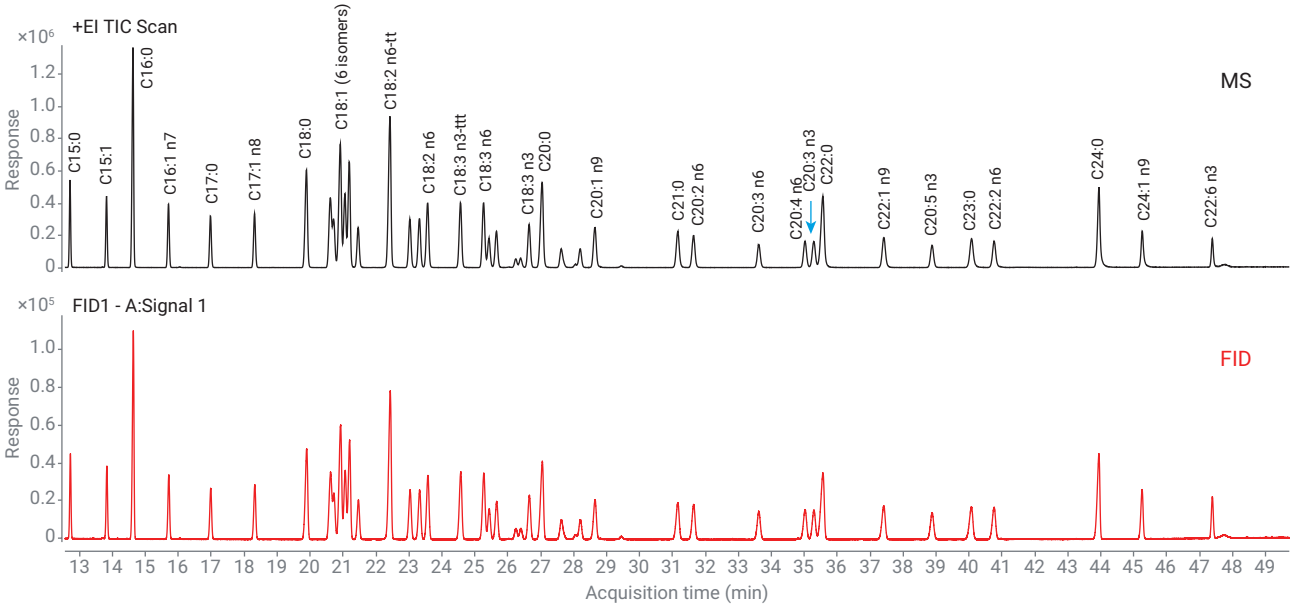

Figure 6. Comparison of TIC (top) and FID trace (bottom) obtained for the analysis of a complex FAME mixture.

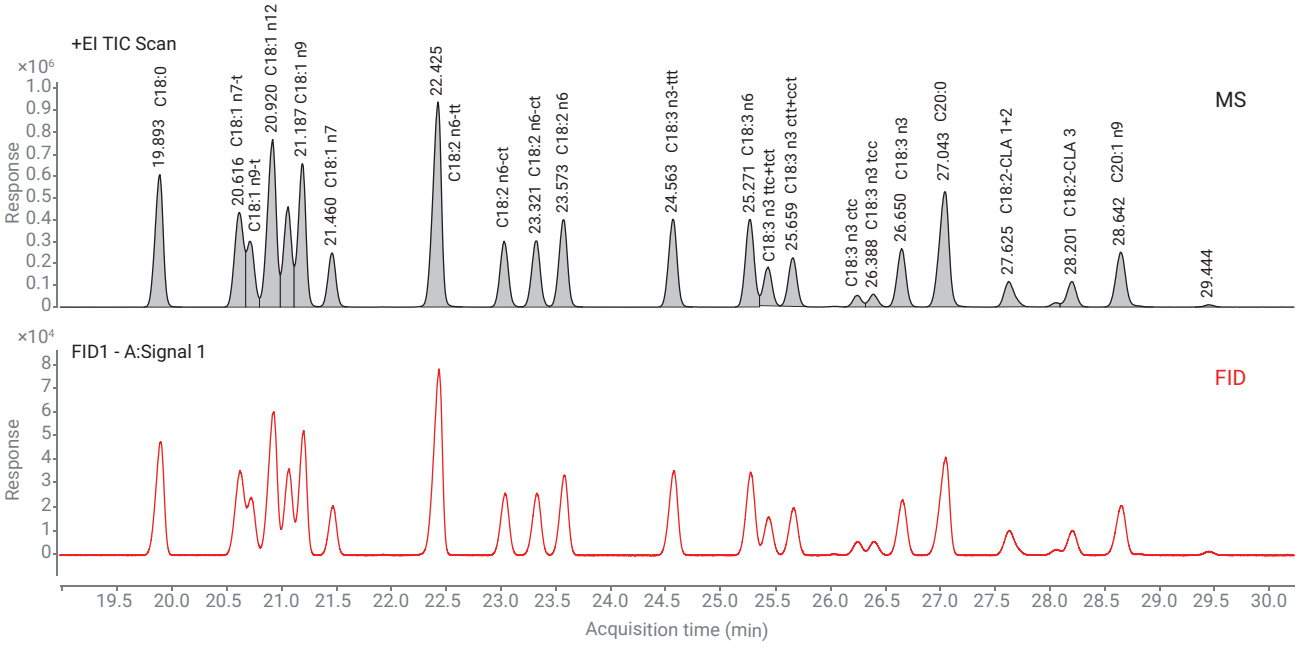

Figure 7. Comparison of TIC (top) and FID trace (bottom) obtained for the analysis of a complex FAME mixture (zoom 19 to 30 minutes).

The TIC profile obtained for the RBC sample is shown in Figure 8. Detailed information is obtained and the important polyunsaturated omega-3 and omega-6 fatty acids are well separated.

Also on this configuration, a repeatability test (n = 6) was performed. Average retention times and peak areas, and the respective standard deviations and relative standard deviations (RSD%), obtained from the TIC traces are summarized in Table 5. As peak area % is typically used for FID results, the average retention times and relative peak areas are reported together with standard deviations and RSD% in Table 6. As for configuration 1, retention time repeatability is typically better than one second standard deviation, and the RSD% on peak areas in the TIC are better than 5% in most cases. The relative standard deviation on relative peak areas, measured by GC/FID, is also better than 5%, except for some trace solutes.

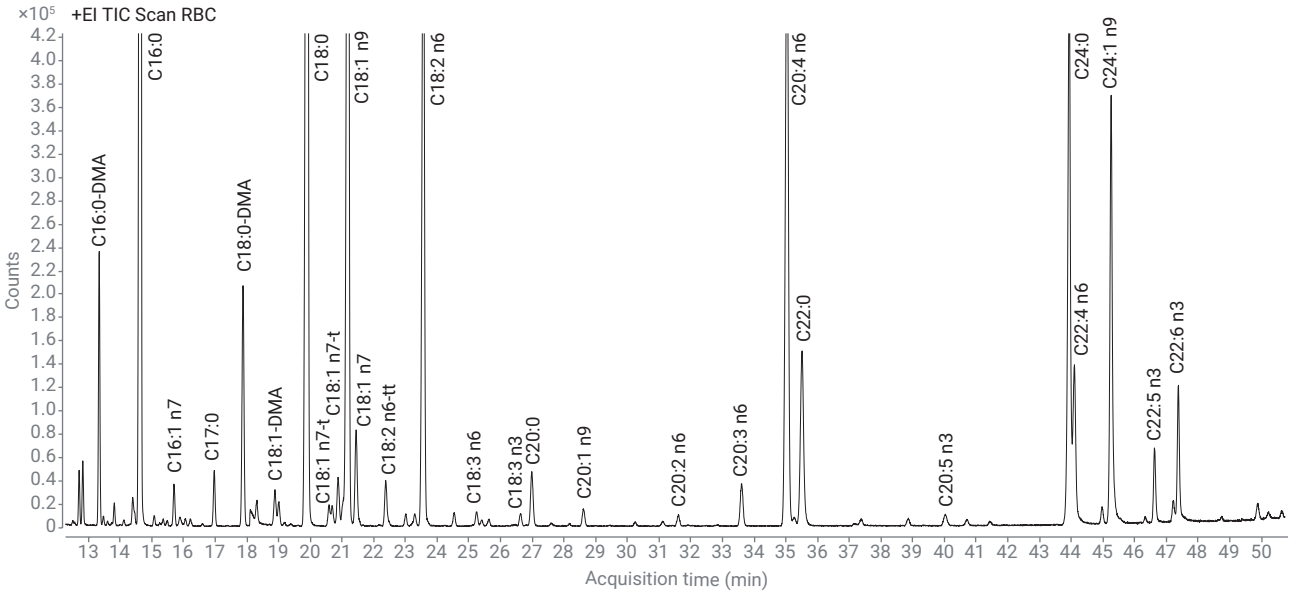

Figure 8. TIC of GC/MS/FID analysis of red blood cell FAMEs using configuration 2.

Table 5. Average TIC retention times and TIC peak areas for main fatty acids detected in RBC using configuration 2. Standard deviations and relative standard deviations (RSD%) (n = 6) are also given.

Solute	Retention Time (min)			Peak Area		
	Mean	Std. Dev.	RSD (%)	Mean	Std. Dev.	RSD (%)
C16:0	14.644	0.004	0.03	10072570	137843	1.37
C16:1 n7	15.697	0.005	0.03	128607	5107	3.97
C17:0	16.967	0.006	0.04	183640	5085	2.77
C18:0	19.905	0.006	0.03	8200917	140241	1.71
C18:1 n9	21.188	0.008	0.04	5844332	158419	2.71
C18:1 n7	21.439	0.010	0.04	397323	29648	7.46
C18:2 tt	22.375	0.011	0.05	205996	6078	2.95
C18:2 n6	23.563	0.010	0.04	3364669	56177	1.67
C20:0	26.983	0.011	0.04	263890	7269	2.75
C20:3 n6	33.602	0.008	0.02	224268	6284	2.80
C20:4 n6	35.036	0.009	0.03	3673852	54901	1.49
C22:0	35.507	0.010	0.03	1045022	29355	2.81
C24:0	43.938	0.006	0.01	2499387	105751	4.23
C22:4 n6	44.099	0.005	0.01	707403	47206	6.67
C24:1 n9	45.259	0.004	0.01	1964329	34899	1.78
C22:5 n3	46.625	0.006	0.01	305509	8644	2.83
C22:6 n3	47.378	0.006	0.01	537861	23373	4.35

Table 6. Average FID retention times and FID relative peak areas for main fatty acids detected in RBC using configuration 2. Standard deviations and relative standard deviations (RSD%) (n = 6) are also given.

Solute	Retention Time (min)			Peak Area		
	Mean	Std. Dev.	RSD (%)	Mean	Std. Dev.	RSD (%)
C16:0	14.651	0.005	0.03	22.79	0.41	1.79
C16:1 n7	15.705	0.006	0.04	0.42	0.03	6.66
C17:0	16.972	0.006	0.03	0.63	0.04	5.88
C18:0	19.910	0.006	0.03	18.87	0.10	0.52
C18:1 n9	21.195	0.007	0.03	13.78	0.10	0.76
C18:1 n7	21.446	0.007	0.03	1.13	0.07	6.04
C18:2 tt	22.378	0.007	0.03	0.87	0.04	4.32
C18:2 n6	23.568	0.008	0.03	8.37	0.03	0.30
C20:0	26.981	0.009	0.03	0.90	0.09	9.74
C20:3 n6	33.598	0.010	0.03	1.10	0.08	7.11
C20:4 n6	35.039	0.009	0.03	10.00	0.41	4.12
C22:0	35.504	0.010	0.03	3.05	0.10	3.13
C24:0	43.935	0.006	0.01	7.39	0.15	1.97
C22:4 n6	44.100	0.006	0.01	2.09	0.11	5.13
C24:1 n9	45.256	0.006	0.01	5.57	0.17	3.06
C22:5 n3	46.626	0.006	0.01	1.32	0.02	1.90
C22:6 n3	47.379	0.005	0.01	1.71	0.08	4.43

Conclusion

Two configurations on the Intuvo 9000 GC were tested for the analysis of FAMEs in biological samples. Using a long, dedicated, highly polar column and carefully selected GC conditions, a very detailed separation of fatty acids could be obtained, including C18:1 *cis-trans* and positional isomers, conjugated linoleic acids (CLAs), and polyunsaturated omega-3 and omega-6 EMFAs. Analysis time was approximately 50 minutes with either method. In configuration 1, postcolumn backflushing is implemented to remove high-molecular-weight, late-eluting compounds, such as cholesterol, from the column. On configuration 2, a Guard Chip temperature program was used to reduce system contamination. As a result, excellent retention time stability and peak area repeatability were obtained on the very polar cyanopropyl column. Over 100 injections were made while maintaining the stability and inertness of the system. Together with the other benefits of the Intuvo 9000 GC, such as easy column installation, Guard Chip maintenance, and instrument diagnostic tools, it is clear that these configurations can be valuable for clinical research of FAMEs.

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