FOOD COMPOSITION AND ADDITIVES

Extension of AOAC Official Method 996.01 to the Analysis of Standard Reference Material (SRM) 1846 and Infant Formulas

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There is currently no official method for the analysis of fatty acids (including *trans* fatty acids) in infant formulas. AOAC Official Method 996.01 for Fat Analysis in Cereal Products was extended to the analysis of milk-based infant formula Standard Reference Material (SRM)1846 to determine its applicability for use with infant formulas. Following the analysis of SRM 1846, 2 infant formulas, one milk-based liquid and one soy-based powdered infant formula, were analyzed for total fatty acid composition. Fatty acid methyl esters were prepared and analyzed by gas chromatography. The results of the analysis of SRM 1846 show that the mean analyzed values were highly reproducible as indicated by low coefficients of variation (CV). The CVs were <5% for the major fatty acids. Mean analyzed values for individual fatty acids in SRM 1846 were within ± 1 standard deviation of the certificate values. The analyzed value for total fat as triglycerides (26.27 ± 0.25%) agreed well with the certificate value (27.1 ± 0.59%). Analyses of infant formulas showed that the concentrations of linoleic acid and fat meet the requirements for such formulas.

Infant formulas are widely used in addition to or as substitutes for human milk. Therefore, the ingredients in the formulas have a great influence on an infant's metabolism and tissue composition (1). Infant formula is the most highly regulated consumer food product on the market today. The Infant Formula Act of 1980, PL-96-359 (2), requires that formula contain minimum and, in some cases, maximum amounts of specific nutrients. Presently, the amount of the added essential fatty acid, linoleic acid (C18:2n-6), in infant formulas seems to be adequate for the healthy growth of infants. However, recent findings (3) indicate that long chain polyunsaturated fatty acids (LCPUFA), particularly arachidonic acid (C20:4n-6, AA) and docosahexaenoic acid (C22:6n-3, DHA), are also important for the growth and development of infants. A recent report by Ratnayake et al. (4) indicated that *trans* fatty acids formed during manufacture of infant formulas may interfere with the formation of these LCPUFA.

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The U.S. Food and Drug Administration has established a series of quality control procedures to ensure that infant formulas contain the necessary nutrients at levels required by the Act (5). Although there are AOAC Official Methods of Analysis for most of the nutrients in infant formulas, there is currently no AOAC Official Method for fatty acids and *trans* fatty acid analysis in infant formulas. For this reason, AOAC Official Method **996.01** (6) was extended to the analysis of milk-based infant formula Standard Reference Material (SRM)1846 in anticipation that this method can be used for the analysis of total fatty acids, including *trans* fatty acids in infant formulas. Following the analysis of SRM 1846, a milk-based liquid infant formula and a soy-based powdered infant formula were analyzed and the results are reported. A soy-based infant formula SRM is not yet available.

METHOD

Principle

Quadruplicate samples of SRM 1846 and duplicate samples of infant formulas were digested with hot HCl and the lipid was extracted with ethyl ether and petroleum ether. The extracts were converted to fatty acid methyl esters (FAME) by methanol in the presence of boron trifluoride. FAMEs of individual fatty acids and *trans* fatty acids were analyzed by gas chromatography (GC). Total fat was calculated as the sum of individual fatty acids expressed as triglycerides in accordance with the nutrition labeling guidelines.

Materials

(**a**) *SRM 1846.*—(National Institute of Standards and Technology [NIST], Gaithersburg, MD.) A milk-based infant formula powder prepared by Analytical Systems Research Corp. (Indianapolis, IN) for NIST. The SRM was manufactured by preparing a spray-dried formula base containing fat (5.25 ± 0.12 g/100 kcal), protein (2.153 ± 0.073 g/100 kcal), carbohydrate (11.10 ± 0.21 g/100kcal), and minerals, and then combining this formula base with dry-blend vitamin premix.

(**b**) *Infant formulas.*—Purchased locally and for term infants. The ingredients of milk-based infant formula are as follows: water, enzymatically hydrolyzed reduced minerals whey protein concentrate (from cow's milk), vegetable oils (palm olein, soy, coconut, high-oleic safflower), lactose, corn

maltodextrin, minerals, and vitamins. Nutrient contents are as follows: protein, 2.4 g/100 kcal; fat, 5.1 g/100 kcal; carbohydrates, 11 g/100 kcal. The ingredients of soy-based infant formula are as follows: corn maltodextrin, vegetable oils (palm olein, soy, coconut, high-oleic safflower), soy protein isolate, minerals, and vitamins. Nutrient contents are as follows: protein, 3.1 g/100 kcal; fat, 4.4 g/100 kcal; carbohydrate, 12 g/100kcal.

Apparatus

(**a**) *Gas chromatograph*.—Equipped with a split injector and flame ionization detector (Hewlett-Packard 5980 series II, Palo Alto, CA).

(**b**) *GC column*.—CP Sil 88 fused-silica capillary column, 100 m × 0.25 mm id and 0.2 μm film thickness (Chrompack, Raritan, NJ).

(**c**) *Analytical balance*.

(**d**) *Extraction tube*.—25 × 200 mm.

(**e**) *Water bath*.—Capable of maintaining temperature at 80 ± 2°C.

(**f**) *Boiling chips*.—Fat free.

(**g**) *Glass wool*.

(**h**) *Steam table*.

(**i**) *Glass funnel*.

(**j**) *Test tube rack*.

(**k**) *Bottle*.—For dispensing acid, l L with 10 mL dispensing bulb or equivalent.

(**l**) *Vortex mixer*.

(**m**) *Reaction flask*.—250 mL Erlenmeyer flat bottom flask.

(**n**) *Condenser*.—Water-cooled reflux condenser, with 40–50 cm jacket and 24/40 joint.

(**o**) *Mixing cylinder*.—50 mL.

Reagents

(**a**) *Ethanol*.

(**b**) *Methanol*.

(**c**) *Hydrochloric acid (HCl)*.—8N (25 + 11, v/v).

(**d**) *Ethyl ether*.—Peroxide free.

(**e**) *Petroleum ether*.—Peroxide free.

(**f**) *Boron trifluoride (BF_3) reagent*.—14% BF_3 in methanol.

(**g**) *Methanolic sodium hydroxide (NaOH) solution*.—0.5N NaOH in methanol.

(**h**) *Sodium chloride (NaCl) solution*.—Saturated aqueous solution (26%, w/v).

(**i**) *n-Heptane*.—GC grade.

(**j**) *Lipid standard solution*.—FAMEs, 100 mg/mL. Mixture of fatty acid standard solution was purchased from Sigma Chemical Co. (St. Louis, MO). To prepare 10 mg/mL mixed FAMEs, break top glass vial, open, and carefully transfer contents to 10 mL volumetric flask. Wash original vial with hexane to ensure complete transfer. Dilute to 10 mL with hexane. The composition and amounts of each fatty acid are shown in Table 1.

(**k**) *Triglyceride internal standard solution*.—C13:0-tritridecanoin, 5.0 mg/mL in hexane. To prepare 5 mg/mL, weigh 250 mg into a 50 mL volumetric flask and dissolve in hexane. Triglyceride internal standard solution is stable up to 1 week if stored in a refrigerator in a sealed amber bottle.

(**l**) *Linoleic acid methyl ester (9, 12 cis/trans isomer mix, 50 mg/mL)*.—To prepare 0.2 mg/mL, dissolve 40 μL *cis/trans* isomer mix in hexane in a 10 mL volumetric flask and fill to

Table 1. Composition of lipid standard [mixture of fatty acid methyl esters (FAMEs)]

Peaks	Chain length	FAMEs	Amount, mg/mL
1	C4:0	Butyric acid	0.4
2	C6:0	Caproic acid	0.4
3	C8:0	Caprylic acid	0.4
4	C10:0	Capric acid	0.4
5	C11:0	Undecanoic acid	0.2
6	C12:0	Lauric acid	0.4
7	C13:0	Tridecanoic acid	0.2
8	C14:0	Myristic acid	0.4
9	C14:1*cis*	Myrostoleic acid	0.2
10	C15:0	Pentadecanoic acid	0.2
11	C15:1*cis*	*cis*-10-Pentadecenoic acid	0.2
12	C16:0	Palmitic acid	0.6
13	C16:1c9	Palmitoleic acid	0.2
14	C17:0	Heptadecanoic acid	0.2
15	C17:1c10	*cis*-10-Heptadecenoic acid	0.2
16	C18:0	Stearic acid	0.4
17	C18:1*trans*	Elaidic acid	0.2
18	C18:1	Oleic acid	0.4
19	C18:2*trans*	Linolelaidic acid	0.2
20	C18:2n-6	Linoleic acid	0.2
21	C20:0	Arachidic acid	0.4
22	C18:3n-6	Gamma-linolenic acid	0.2
23	C20:1	*cis*-11-Eicosenoic acid	0.2
24	C18:3n-3	Linolenic acid	0.2
25	C21:0	Heneicosanoic acid	0.2
26	C20:2	Eicosadienoic acid	0.2
27	C22:0	Behenic acid	0.4
28	C20:3n-6	Eicosatrienoic acid	0.2
29	C22:1n-9	Erucic acid	0.2
30	C20:3n-3	Eicosatrienoic acid	0.2
31	C20:4n-6	Arachidonic acid	0.2
32	C23:0	Tricosanoic acid	0.2
33	C22:2n-6	Docosadienoic acid	0.2
34	C24:0	Lignoceric acid	0.4
35	C20:5n-3	Eicosapentaenoic acid	0.2
36	C24:1	Nervonic acid	0.2
37	C22:6n-3	Docosahexaenoic acid	0.2

10 mL mark with hexane. The composition of the mixture is as follows (mg/mL): tt-0.10; ct-0.04; tc-0.04; cc-0.02.

(**m**) *Linolenic acid methyl ester (9, 12, 15 cis/trans isomer mix, 25 mg/mL).*—To prepare 0.4 mg/mL, dissolve 80 μL *cis/trans* isomer mix in hexane in a 5 mL volumetric flask and fill to 5 mL mark with hexane. The composition of the mixture is as follows (mg/mL): ttt-0.12; ttc-0.06; tct-0.06; ctt-0.06; cct-0.03; ctc-0.03; tcc-0.03; ccc-0.01.

Sample Hydrolysis

(**a**) Equilibrate water bath to 80°C.

(**b**) Weigh ca 1 ± 0.001 g SRM 1846 into a test tube. Repeat this with 3 different packets containing SRM 1846. Add 1 mL triglyceride internal standard solution to each tube and vortex mix. Weigh the same amounts (in duplicate) of infant formulas into test tubes. Proceed as above.

(**c**) Add 2 mL ethanol, mix thoroughly to ensure adequate wetting of all particles, and then add 10 mL of 8N HCl. Cover with caps.

(**d**) Vortex mix tubes thoroughly to moisten the contents. Place tubes in 80°C water bath for 40 min.

(**e**) Remove the test tubes from water bath and immediately add 10 mL ethanol, vortex mix, and then cool in water bath to room temperature.

Sample Extraction

(**a**) Transfer entire contents of each test tube into 250 mL separatory funnel. Rinse tubes with 15 mL ethyl ether, and

Table 2. Theoretical factors for conversion of FAMEs to their corresponding triglycerides (F_{TG}) and corresponding free fatty acids (F_{FA}; from ref. 7)

	Molecular weight, g/mol			Conversion factor	
Description	Fatty acid	FAME	Triglyceride	F_{TG}[a]	F_{FA}[b]
C6:0	116.16	130.19	389.53	0.9897	0.8923
C8:0	144.22	158.24	470.70	0.9915	0.9114
C10:0	172.27	186.30	554.86	0.9928	0.9247
C12:0	200.32	214.35	639.02	0.9937	0.9346
C13:0	214.35	228.38	681.10	0.9941	0.9386
C14:0	228.38	242.41	723.18	0.9945	0.9421
C14:1*cis*	226.36	240.39	717.14	0.9944	0.9416
C15:0	242.41	256.43	765.26	0.9948	0.9453
C16:0	256.43	270.46	807.35	0.9950	0.9481
C16:1*cis*	254.42	268.44	801.30	0.9950	0.9477
C17:0	270.46	284.49	849.43	0.9953	0.9507
C17:1*cis*	268.44	282.47	843.38	0.9952	0.9503
C18:0	284.49	298.51	891.51	0.9955	0.9530
C18:1*trans*	282.47	296.50	885.46	0.9955	0.9526
C18:1*cis*	282.47	296.50	885.46	0.9955	0.9527
C18:2*trans*	280.45	294.48	879.41	0.9954	0.9524
C18:2	280.45	294.48	879.41	0.9954	0.9524
C20:0	312.54	326.57	975.67	0.9959	0.9570
C18:3n-6	278.44	292.47	873.36	0.9954	0.9520
C20:1*cis*	310.52	324.55	969.62	0.9959	0.9568
C18:3*trans*	278.44	292.47	873.36	0.9954	0.9520
C18:3n-3	278.44	292.47	873.36	0.9954	0.9520
C22:0	340.59	354.62	1059.83	0.9962	0.9604
C20:4	304.48	318.50	951.48	0.9958	0.9560
C24:0	368.65	382.68	1144.00	0.9965	0.9633
C20:5	302.46	316.49	945.43	0.9958	0.9557
C24:1	366.63	380.66	1137.95	0.9965	0.9632

[a] F_{TG} = mol wt of triglycerides/(mol wt of FAME × 3).

[b] F_{FA} = mol wt of free fatty acids/mol wt of FAME.

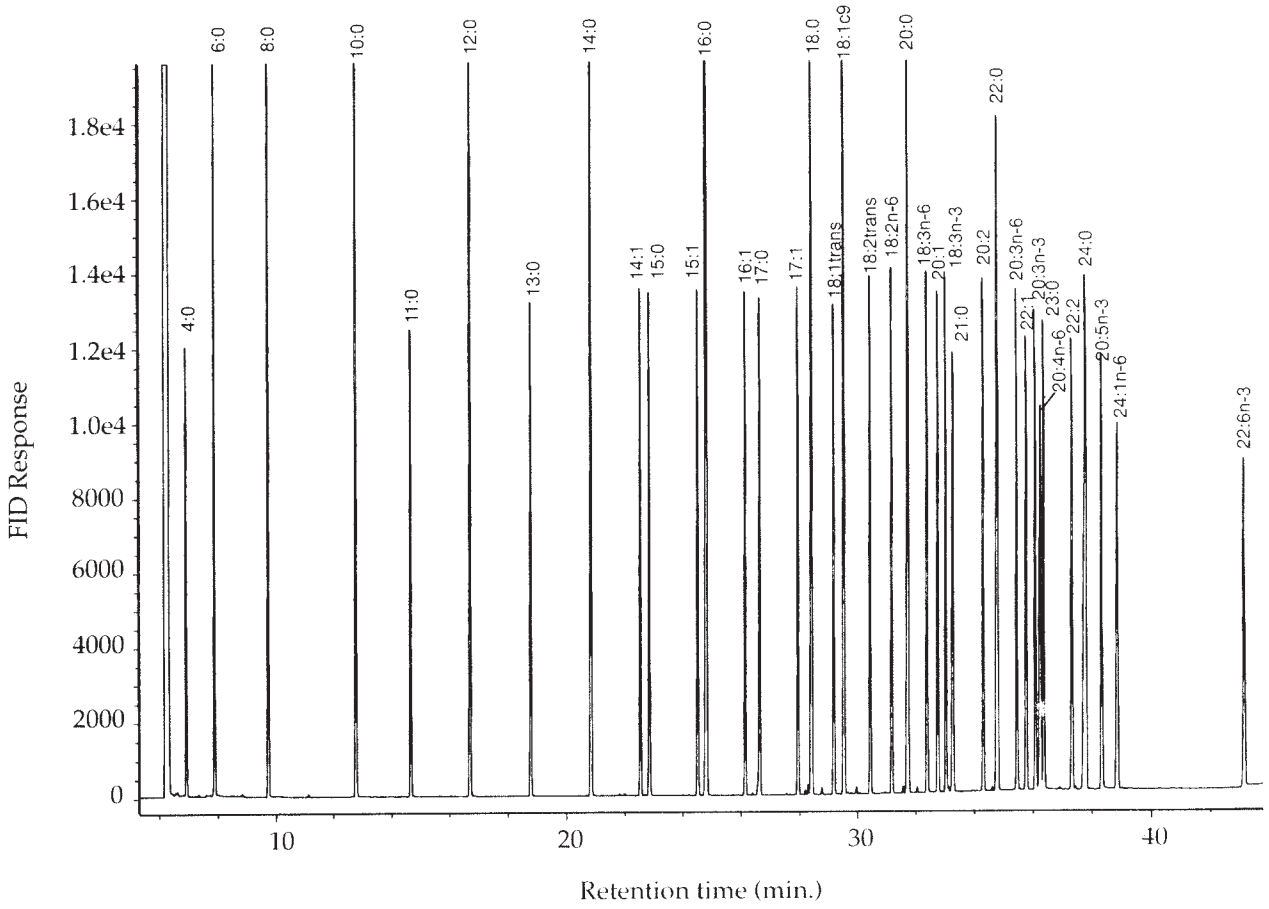

Figure 1. Chromatogram from GC analysis of standard FAMEs mixture.

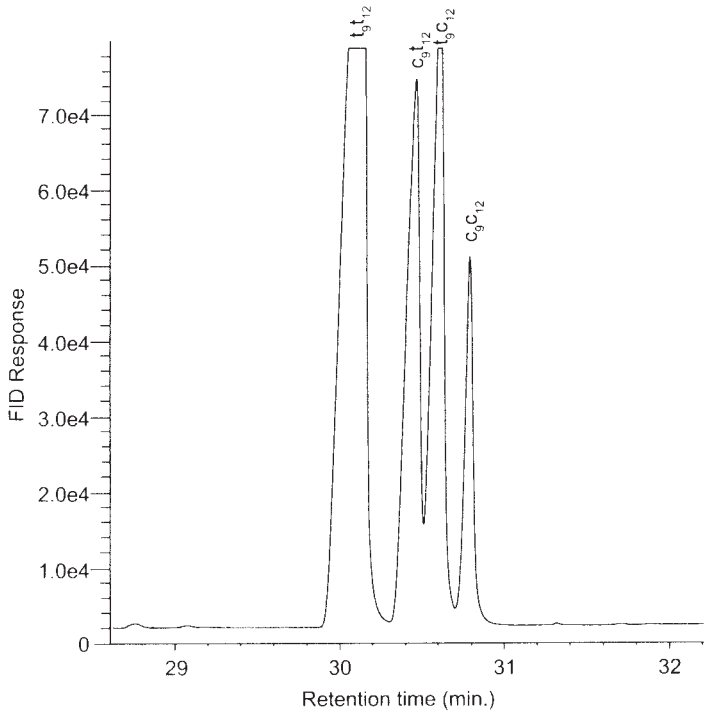

Figure 2. Chromatogram from GC analysis of standard *cis/trans* linoleic acid methyl ester.

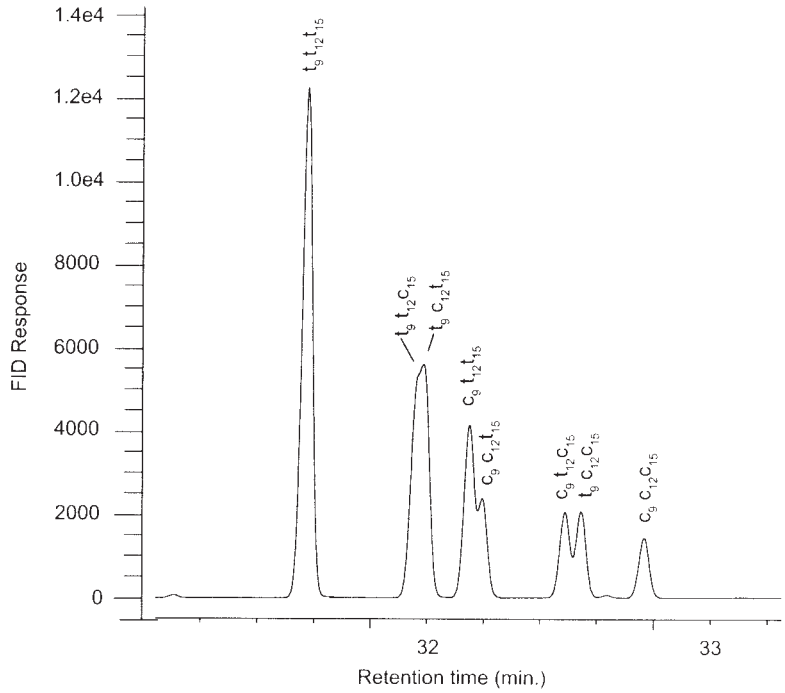

Figure 3. Chromatogram from GC analysis of standard *cis/trans* linolenic acid methyl ester.

Table 3. Fatty acid composition of SRM 1846, mg/g sample[a]

	Analyzed values									
Fatty acids	1	2	3	4	Mean	SD (+/–)	CV, %	Reference values	SD (+/–)	Rec., %
C6:0	0.56	0.54	0.70	0.66	0.62	0.08	12.56	0.63[b]	NR[c]	98
C8:0	5.41	5.35	5.61	5.43	5.45	0.11	2.04	6.0[b]	NR	91
C10:0	4.80	4.68	4.89	4.77	4.78	0.09	1.83	4.7[b]	NR	102
C12:0	38.31	36.83	38.80	37.78	37.93	0.84	2.22	36.5	5.6	104
C14:0	16.08	15.39	16.20	15.82	15.87	0.36	2.27	15.4	1.3	103
C15:0	0.09	0.07	0.10	0.07	0.08	0.02	18.68	0.11[b]	NR	72
C16:0	29.85	28.39	30.07	29.48	29.45	0.75	2.53	29	1.5	102
C17:0	0.24	0.22	0.23	0.22	0.23	0.01	3.99	0.24[b]	NR	96
C18:0	29.40	28.01	29.75	29.32	29.12	0.76	2.62	28.4	1.4	103
C20:0	0.84	0.80	1.06	0.95	0.91	0.12	12.88	0.88	0.11	103
C22:0	0.601	0.604	0.575	0.575	0.59	0.02	2.70	0.57	0.08	104
C24:0	0.433	0.414	0.391	0.393	0.41	0.02	4.85	0.39[b]	NR	105
C16:1	0.18	0.18	0.18	0.18	0.18	0.00	1.40	0.21	0.03	86
C18:1	48.55	48.96	49.40	52.54	49.86	1.82	3.65	60.0	1.5	102
C18:1*trans*	52.78	52.96	53.42	48.13	51.82	2.48	4.78	40.0	5.4	
C22:1	0.12	0.13	0.12	0.11	0.12	0.01	7.20	NR		
C18:2n-6	33.80	31.84	33.98	33.23	33.21	0.97	2.93	35.0	4.0	95
C18:3n-3	0.93	0.87	0.92	0.89	0.90	0.02	2.74	0.98	0.11	92
Sum	263.03	265.39	266.00	260.40	263.71	2.55	0.97	NR	NR	NR
% Fat as triglycerides	26.19	26.42	26.50	25.95	26.27	0.25	0.94	27.1	0.59	97

[a] CV, % = (SD/mean) × 100.
[b] Information values.
[c] NR = not reported.

transfer to separatory funnels. Repeat this step with another 15 mL ether.

(**b**) Shake separatory funnel gently for 1 min by partial inversions. Release pressure often by removing stopper. (*Note:* If samples stick to sides, swirl the separatory funnels.)

(**c**) Repeat steps (**a**) and (**b**) with all test samples.

(**d**) Add 30 mL petroleum ether and shake well for 1 min.

(**e**) Let stand for 5 min and separate bottom layer from ether layer and transfer ether layer into another clean separatory funnel.

(**f**) Add 30 mL mixture of ethyl ether and petroleum ether (1 + 1) to bottom layer and shake for 1 min.

(**g**) Allow to stand for 5 min and transfer top ether layer to the fraction in step (**e**). Add another 30 mL ether–petroleum ether mixture (1 + 1) to bottom layer, shake for 1 min, and let stand for 5 min. Add top ether layer to the fraction in step (**e**).

(**h**) Filter the pooled ether layer through fat-free glass wool packed firmly enough in stem of funnel to allow free passage of ether into 125 mL Erlenmeyer flat bottom flasks containing boiling chips.

(**i**) In a ventilated hood, evaporate ether to near dryness on a steam table under a stream of nitrogen.

Sample Methylation

(**a**) Add 10 mL methanolic NaOH to the extracted fat from step (**i**), *Sample Extraction*. Attach Erlenmeyer flasks to a water-cooled condenser, seal joint with methanol, and heat. Reflux for 10 min.

(**b**) Add 10 mL BF_3 reagent through top of condenser and reflux for additional 5 min.

(**c**) Add 10 mL *n*-heptane through top of condenser. Continue to reflux for additional 1 min.

(**d**) Remove Erlenmeyer flask from heat. Allow flask to cool (*Note*: Allow flask to cool to room temperature before removing from condenser.)

Transfer the entire contents into a 50 mL glass cylinder. Rinse the flask with 5 mL NaCl solution. Rotate flask gently several times before transferring washes to the measuring cylinder. Mix contents of the tube well by shaking gently and allow layers to separate. Upper layer which contains the fatty acid methyl esters in *n*-heptane is used for fatty acid analysis.

Chromatographic Conditions

The following operating conditions were used: Temperature, injector 250°C and detector 250°C; temperature was programmed from 120°C for 5 min and increased to final temperature of 240°EC at the rate of 4°C/min for 30 min. It was kept at the final temperature for 25 min. Carrier gas (hydrogen) flow rate: 1 mL/min. Split ratio 20:1. Hydrogen linear velocity 28.5 cms/s at 120°C.

Calculations

(**a**) *GC standardization/calibration*.—Calculate the response factor (Ri) for each individual fatty acid (i) as described in ref. 6, according to the following equation:

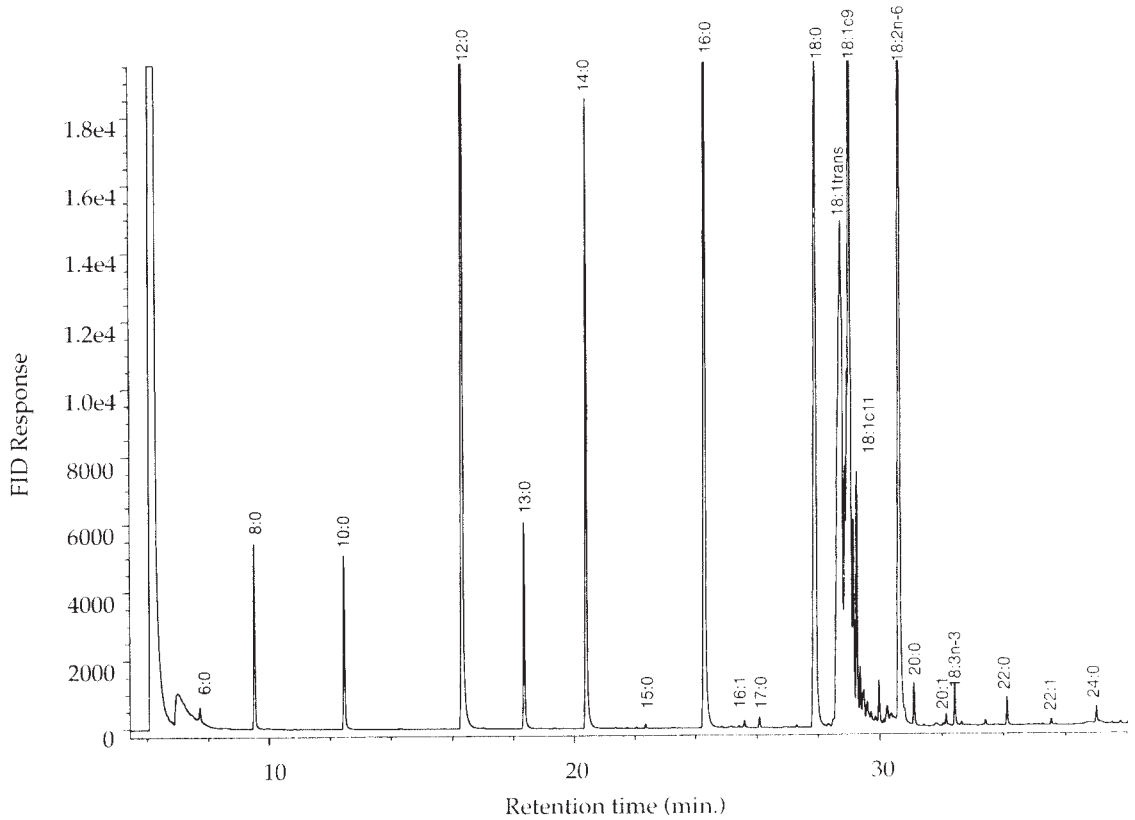

Figure 4. Chromatogram from GC analysis of SRM 1846.

$$Ri = \frac{(Ai)(Wt13:0)}{(A13:0)(Wti)} \quad (1)$$

$$Fi = \frac{(Ai)(Wt13:0)}{(A13:0)(Ri)} \quad (2)$$

where Ai = peak area of individual FAME in the standard, Wt13:0 = weight (mg) C13:0 in the standard, A13:0 = peak area of C13:0 FAME in the standard, and Wti = weight (mg) of individual FAME in the standard.

(**b**) *GC calculations*.—Calculate the amount of individual fatty acid (Fi) in each test sample (as corresponding methyl ester) according to the following equation:

where Ai = peak area of the individual fatty acid in test sample as FAME; Wt13:0 = weight (mg) of internal standard C13:0 in test sample; A13:0 = peak area of internal standard, C13:0 in test sample; and Ri = response factor for each fatty acid.

Calculate the amount of individual fatty acid as the corresponding triglyceride, Fi_{TG} according to the following equation:

Table 4. Fatty acid composition of milk-based liquid and soy-based powdered infant formulas (amount %)

	Milk-based liquid infant formula				Soy-based powdered infant formula			
Fatty acids	1	2	Avg.	SD(+/–)	1	2	Avg.	SD(+/–)
C6:0	0.58	0.14	0.36	0.31	0.10	0.12	0.11	0.01
C8:0	1.29	1.35	1.32	0.04	1.32	1.33	1.33	0.01
C10:0	1.24	1.27	1.26	0.02	1.14	1.16	1.15	0.01
C12:0	9.02	9.09	9.06	0.05	9.04	9.24	9.14	0.14
C14:0	4.36	4.39	4.38	0.02	4.08	4.17	4.13	0.06
C15:0	0.08	0.08	0.08	0.00	0.03	0.03	0.03	0.00
C16:0	23.11	23.30	23.21	0.13	22.58	22.99	22.79	0.29
C17:0	0.12	0.12	0.12	0.00	0.08	0.08	0.08	0.00
C18:0	4.27	4.29	4.28	0.01	3.89	3.96	3.93	0.05
C20:0	0.03	0.33	0.18	0.21	0.33	0.34	0.34	0.01
C22:0	0.42	0.06	0.24	0.25	0.42	0.42	0.42	0.00
C24:0	0.14	0.14	0.14	0.00	0.12	0.12	0.12	0.00
Total SFA	44.66	44.56	44.61	0.07	43.13	43.96	43.55	0.59
C14:1	0.03	0.03	0.03	0.00	0.03	0.02	0.03	0.01
C16:1	0.20	0.21	0.21	0.01	0.29	0.02	0.16	0.19
C17:1	0.05	0.05	0.05	0.00	0.03	0.03	0.03	0.00
C18:1	32.69	32.62	32.66	0.05	31.78	31.68	31.73	0.07
C20:1	0.16	0.16	0.16	0.00	0.20	0.15	0.18	0.04
C24:1	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00
Total MUFA	33.15	33.09	33.12	0.04	32.33	31.90	32.12	0.30
C18:2n-6	19.25	19.36	19.31	0.08	21.25	21.31	21.28	0.04
C18:3n-3	2.04	2.05	2.05	0.01	2.32	2.30	2.31	0.01
C18:3n-6	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.00
C20:4n-6	0.06	0.06	0.06	0.00	0.00	0.00	0.00	0.00
C20:5n-3	0.06	0.06	0.06	0.00	0.00	0.00	0.00	0.00
Total PUFA	21.43	21.54	21.49	0.08	23.58	23.62	23.60	0.03
C18:1*trans*	0.40	0.32	0.36	0.06	0.28	0.18	0.23	0.07
C18:2*trans*	0.25	0.25	0.25	0.00	0.23	0.24	0.24	0.01
C18:3*trans*	0.07	0.07	0.07	0.00	0.15	0.15	0.15	0.00
Total *trans*	0.72	0.64	0.68	0.06	0.66	0.57	0.62	0.06
Fat, g/100 kcal	5.00	5.00	5.00	0.00	4.45	4.36	4.40	0.06
Linoleic acid, mg/100 kcal	958.00	959.00	958.50	0.71	947.00	931.00	939.00	11.31
Ratio n-6/n-3	9.20	9.60	9.40	0.28	9.10	9.20	9.20	0.07

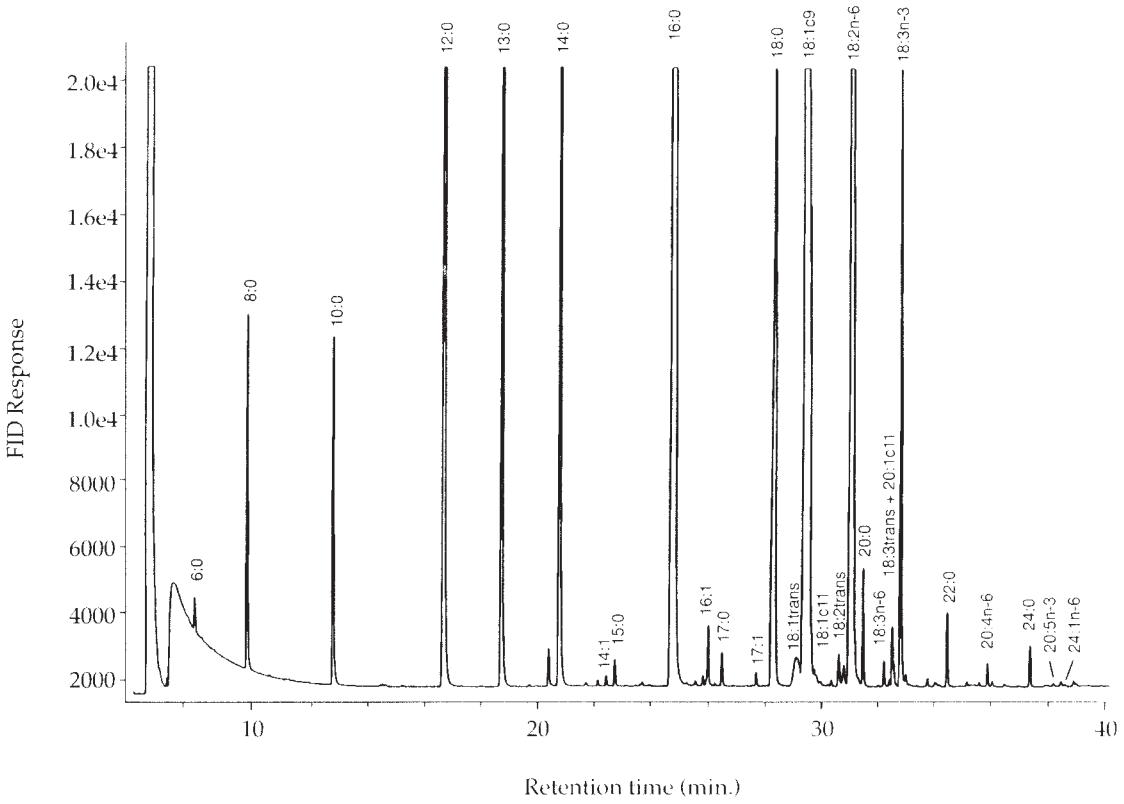

Figure 5. Chromatogram from GC analysis of a milk-based liquid infant formula.

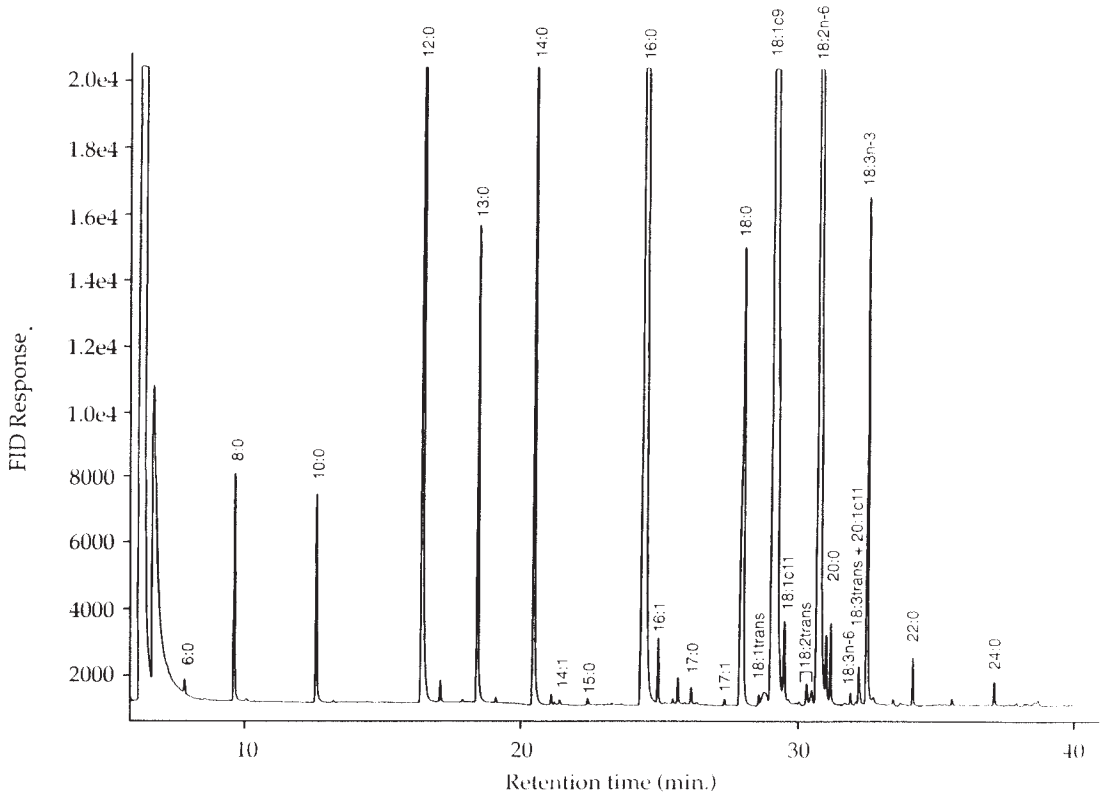

Figure 6. Chromatogram from GC analysis of a soy-based powdered infant formula.

$$Fi_{TG} = (Fi)(F_{TG}) \quad (3)$$

where F_{TG} = theoretical conversion factors from FAMEs to their corresponding triglycerides (Table 2, F_{TG}).

Calculate the amount of individual fatty acid as the corresponding fatty acids, Fi_{FA} according to the following equation:

$$Fi_{FA} = (Fi)(F_{FA}) \quad (4)$$

where F_{FA} = theoretical conversion factors for conversion of FAMEs to their corresponding fatty acids (Table 2, F_{FA}).

Calculate the amount of total fat (sum of all fatty acids) in each sample expressed as triglycerides according to the following equation:

$$\text{Total fat, \%} = [\Sigma\ Fi_{TG}/\text{wt sample (g)}] \times 100 \quad (5)$$

Calculate the amount of saturated fat (expressed as sum of all saturated fatty acids: C6:0 to C24:0) in each sample according to the following equation:

$$\text{Saturated fat, \%} = [\Sigma\ \text{saturated fat}\ Fi_{FA}/\text{wt sample (g)}] \times 100 \quad (6)$$

Calculate the amount of monounsaturated fat (expressed as fatty acids: C14:1, C16:1, C17:1, C18:1, *cis* and *trans*, C20:1 and C24:1) according to the following equation:

$$\text{Monounsaturated fat, \%} = [\Sigma\ \text{monounsaturated fat}\ Fi_{FA}/\text{wt sample (g)}] \times 100 \quad (7)$$

Calculate the amount of polyunsaturated fat (expressed as fatty acids:C18:2, *cis* and *trans*, C18:3, *cis* and *trans*, C20:4 and C20:5) according to the following equation:

$$\text{Polyunsaturated fat, \%} = [\Sigma\ \text{polyunsaturated fat}\ Fi_{FA}/\text{wt sample (g)}] \times 100 \quad (8)$$

Calculate the amount of *trans* fat (expressed as fatty acids C18:1 *trans*, C18:2 *trans*, and C18:3 *trans*) according to the following equation:

$$\textit{trans}\ \text{Fat, \%} = [\Sigma\ \textit{trans}\ \text{fat}\ Fi_{FA}/\text{wt sample (g)}] \times 100 \quad (9)$$

Results and Discussion

The composition of the FAMEs in the standard fatty acid mixture is shown in Table 1 and a gas chromatogram of FAMEs of the same standard mixture is shown in Figure 1. Gas chromatograms of FAMEs of *cis/trans* mixture of linoleic acid and linolenic acid are shown in Figures 2 and 3, respectively. Analyzed values for individual fatty acids in SRM 1846 are shown in Table 3 and a gas chromatogram of FAMEs is shown in Figure 4. The values were highly reproducible as indicated by low coefficients of variation (CV). The CVs were <5% for the major fatty acids. Mean analyzed values for fatty acids in SRM 1846 were within ± 1 standard deviation (SD) of the reference values with the following exceptions: analyzed values for C18:1 were significantly lower (analyzed value, 49.86 ± 1.8 mg/g versus reference value, 60.0 ± 1.5 mg/g) and those for C18:1*trans* were significantly higher (analyzed value, 51.82 ± 2.5 mg/g versus reference value, 40.0 ± 5.4 mg/g) than values reported in the Certificate of Analysis for SRM 1846. These differences may be due to the use of different chromatographic columns and parameters during the analyses used to obtain the certificate values. Under different analytical conditions, some of the C18:1*trans* may coelute with C18:1, resulting in a higher value for the latter. The analyzed value of total fat as triglycerides (26.27 ± 0.25 g/100 g) agreed well with the Certificate of Analysis value of 27.1 ± 0.59 g/100 g. The analyzed values for major fatty acids and total fat are between 91–105% of the reported values.

The analyzed values for fatty acids in a milk-based liquid and a soy-based powdered infant formulas are shown in Table 4. A chromatogram from a GC analysis of a milk-based infant formula is shown in Figure 5 and a chromatogram from a GC analysis of soy-based infant formula is shown in Figure 6. The results show that there were no significant differences in fatty acid composition between the 2 infant formulas. All 3 C18*trans* isomers (18:1*trans*, 18:2*trans*, and 18:3*trans*) are present in both formulas. In milk-based liquid infant formula, total *trans* fatty acid content (mean ± SD) was 0.68 ± 0.06%, compared with 0.62 ± 0.06% in soy-based powdered infant formula. In milk-based liquid infant formula, the analyzed value for total fat was 5.0 ± 0.0 g/100 kcal and that for soy-based powdered infant formula was 4.40 ± 0.06 g/100 kcal. These analyzed values were 98 and 101% of the values provided on the product labels (5.1 and 4.4 g/100 kcal for milk-based and soy-based formulas, respectively). In milk-based liquid infant formula, the analyzed value for 18:2n-6 content was 958.5 ± 0.71 mg/100 kcal, while that for soy-based powdered infant formula was 939 ± 11.31 mg/100 kcal (mean ± SD). Both infant formulas satisfy the requirements for a minimum of 300 mg/100 kcal linoleic acid and 3.3–6.0 g/100 kcal fat in such formulas (8).

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