GC-FID- and Acyl Carbon Number-Based Determination of Characteristic Groupings of Complex Triglyceride (Benefat S and Other) Mixtures

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Techniques for the gas and liquid chromatographic separation of complex mixtures of triglycerides have evolved over the past two decades, as reviewed in detail by Huang et al. (*J. Agric. Food Chem.* **1995**, *43*, 1834−1844; *J. Agric. Food Chem.* **1997**, *45*, 1770−1778). A novel method for the quantitative partitioning of complex mixtures of triglycerides into functionally related groups is developed and applied to a low-calorie triglyceride mixture [namely, Benefat S or Salatrim plus mid-chain ($C_{6,8,10,12}$) fatty acids]. The method is based on a nonlinear calibration of retention times (RTs) of a suite of standard triglycerides on their acyl carbon numbers [(ACNs), the sum of all the acyl carbon atoms in a given triglyceride] to estimate all of the intermediate ACNs (from 6 to 66). With the calibrated ACN scale and identifications of some components of a complex mixture's composition, ACN-based partitions were established and a Benefat S−triglyceride chromatogram was partitioned into seven functionally related groups. This method is provisional in the sense that it would typically be employed when the identifications of many components of a complex, homologous series were unknown, yet functionally related groups needed to be quantified. This method has proven to be particularly useful in the intercalibration of research laboratories with production facility laboratories during complex (∼50−90 compounds) and large-scale (∼20 ton) syntheses because of the high reproducibility of the ACN-based partitioning of complex chromatograms. This carbon number and statistically based method can be generally applicable to other complex mixtures of organic compounds and is readily adaptable to laboratory intercalibration efforts.

Keywords: *Triglycerides; complex mixtures; quantitation; partitioning; acyl carbon numbers*

INTRODUCTION

In the quantification of the components of a complex chromatographic mixture, one would ideally prefer to identify all of the individual components and, with their relative response factors [(RRFs) detector response of a given compound per unit mass relative to that of a standard], calculate their individual or grouped masses. Typically, however, pure standards may not exist for all members of complex mixtures, thereby preventing measurement of individual compounds' RRFs and calculation of individual or grouped masses. As a practical measure to quantify selected groups of compounds, a provisional method based on the separation of functionally related compounds delineated by a combination of acyl carbon numbers (ACNs) and a nonlinear statistical technique is developed here.

Benefat is synthesized by the interesterification of hydrogenated vegetable oil and mid- and short-chain triglycerides. The finished product is a mixture of short-, mid-, and long-chain fatty acids esterified onto a glycerol backbone (Klemann et al., 1994; Smith et al., 1994; Softly et al., 1994). For example, Benefat S shortening is a complex mixture of ∼50−90 triglycerides that is categorized and quantified here for chemical and commercial purposes.

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A suite of commercially available homogeneous triglycerides (2-2-2 etc.) and a statistical method for the reconstruction of retention times (RTs) of various ACNs permit a quantitative estimate of the mass percent distribution of complex mixtures of triglycerides (namely, Benefat S shortening). The RTs and RRFs of a suite of commercially available triglycerides (namely, 2-2-2 to 22-22-22) permit a statistical relationship between RT and ACN to be constructed (in this case, a third-order polynomial). Using known ACNs from 6 to 66, statistically estimated RTs are reconstructed for all of the intermediate compounds. In this case, seven groups of compounds defined by their ranges of ACN are then calculated. The sum of their group area is converted to mass per group, and then the mass percent (per group) is calculated. This analysis yields a concise (∼1 order of magnitude less complicated) summary of a complex mixture that has been successfully applied in monitoring syntheses at pilot and plant scales, as well as serving as quantitative indices for commercial certificates of analysis.

Test products consisting of various complex mixtures of compounds are produced, which must be qualitatively subdivided and quantified. The goal of the present procedure is to identify and quantify the ∼60 triglycerides in the present Benefat S shortening. At the time that this project was undertaken, however, only 8 authentic standards were available, so this iterative method was developed to identify and categorize the remaining compounds by ACN. The method accepts that individual triglycerides elute in order of their ACNs

10.1021/jf990059t CCC: $19.00
Published on Web 02/05/2000

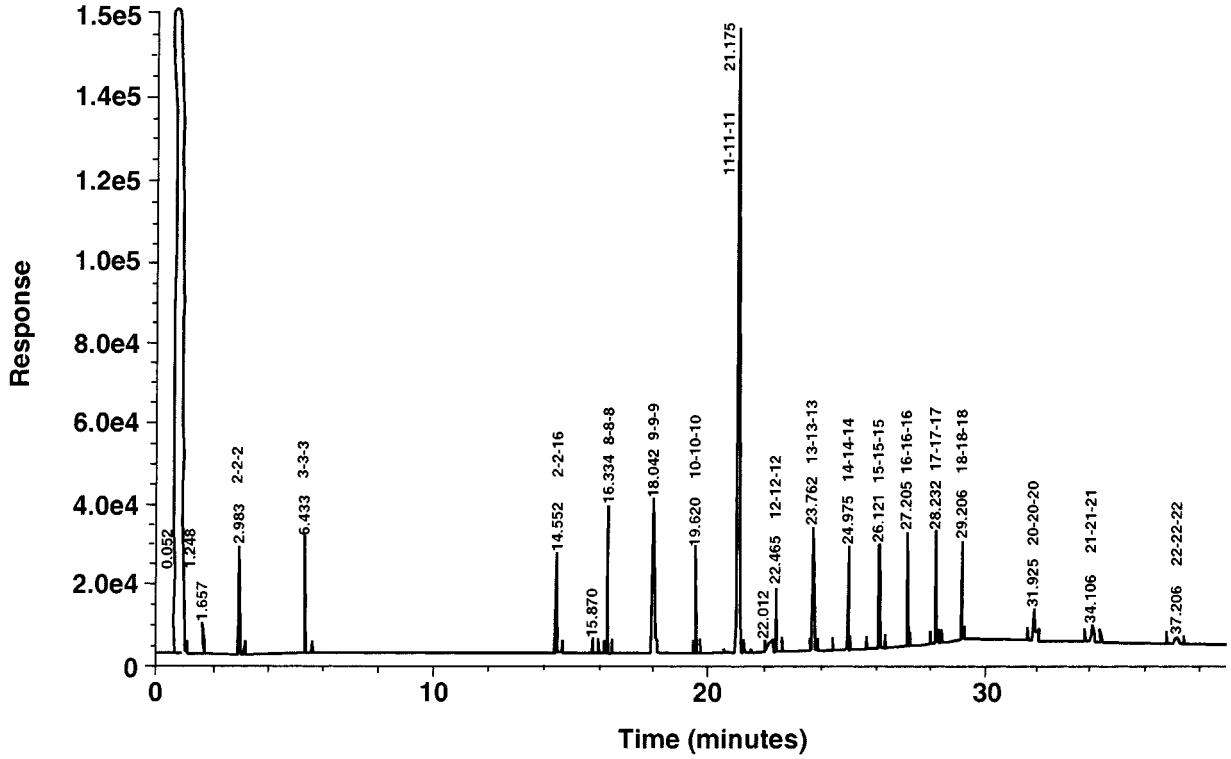

Figure 1. Gas chromatogram of a mixture of predominantly homogeneous triglycerides (2-2-2 to 22-22-22; cf. Table 1) separated using the comprehensive method described under Materials and Methods. This procedure employs a DB-5HT, 5% phenyl−95% methylpolysiloxane, 0.32 mm i.d. × 0.1 μm GC column (J&W Scientific) with the following temperature program: 85 °C (0 min), ramped at 10 °C/min to 375 °C (10 min).

(Huang et al., 1994) much as *n*-alkanes elute in the order of total carbon number [compare with Kovat's retention index of *n*-alkanes (e.g., Karasek and Clement (1988))]. Thus, an iterative method based on the regression on RT upon ACN and systematic prediction of RT for all ACNs was developed.

Specifically, the present ACN-based statistical procedure applies to Benefat process streams generated from triacetylglycerol, tripropionylglycerol, mid-chain triglycerides, and hydrogenated soybean oil. This includes the analyses of distillates, residues, and finished products. More generally, this ACN-based statistical procedure for the subdivision of compound groups can be adapted to many types of chromatographable mixtures.

MATERIALS AND METHODS

Chemicals and Reagents. Used as a solvent, toluene (ACS) was from Fisher Scientific (T324-4).

The standard compounds used here were from Sigma Chemical Co. (St. Louis, MO) (triacetin, tripropinin, tributyrin) and from Nu Chek Prep (Elysian, MN) (diacetylpalmitoylglycerol, trioctanoin, trianoin, triundecanoin, tridodoceanoin, tritridecanoin, tritetradecanoin, tripentadecanoin, trihexadecanoin, triheptadecanoin, tristearintrinonadecanoin, triheneicocosoin, and tridocosoanoin).

Samples. The sample used in this study is a prototype Benefat S sample [Salatrim plus mid-chain ($C_{6,8,10,12}$) fatty acids] that became the standard reference material for the present study of complex triglyceride mixtures.

Gas Chromatographic Equipment. Gas chromatographic analysis was performed on a Hewlett-Packard (HP) 5890 series II Plus gas chromatograph (GC) equipped with an HP 3365 series II chromatographic software data system. The GC was equipped with a flame ionization detector (FID) held at 380 °C, a cool on-column injector, an HP7673 automatic sampler equipped with a 5 μL syringe, an HP 1 m × 0.32 mm i.d. deactivated fused silica retention gap with capillary column connectors, and a 15 m × 0.32 mm i.d. fused silica capillary column (J&W Scientific Inc.) coated with a 0.1 μm DB5-HT (5%-methyl−95%-methylpolysiloxane) liquid phase. Chromatographic separation was achieved with high-purity helium as the carrier gas, toluene as the solvent, and an injection volume of 0.5 μL.

Chromatographic Separations. The following temperature programs are used to achieve chromatographic separations for triglyceride mixtures of various complexities: start at 85 °C, increase to 375 °C at 10 °C/min, and hold at 375 °C for 10 min (total time = 48.83 min; helium pressure at 6.5 psi, constant flow). For less complicated triglyceride mixtures, the following temporally optimized method was used: start at 90 °C, increase to 375 °C at 27 °C/min, and hold at 375 °C for 0.44 min (total time = 16.0 min; helium pressure at 8.5 psi, constant flow).

The use of relative retention times (RRTs) rather than absolute RTs would assist in even further minimizing potential errors in the ACN versus RT relationships developed here. However, with the high level of reproducibility in RTs with the system presented here, the use of RRTs detracted from the one-to-one relationships of chromatograms to ACN versus RT graphs.

Estimation of RRFs of Benefat S Triglycerides from Commercial Standards. This estimation was performed as follows:

(1) Prepare a standard reference solution of 0.3 ± 0.1 mg/g of triundecanoin (11-11-11) in toluene.

(2) Prepare individual triglyceride (2-2-2 to 22-22-22) standard solutions at ~0.3 ± 0.1 mg/g toluene concentrations using the standard reference solution as the diluent.

(3) Dilute the triglyceride standard solution by a factor of 2 in toluene to produce 50% diluted standard solution for calibration of RRFs. Analyze by GC. [It is recommended that the linearity of response of a given GC should be tested with some dilution experiments. One can thereby better judge how many dilutions are necessary in determining response factors (RFs) for given compounds. Experience has shown that GC

Table 1. Example of Mixture of Predominantly Homogeneous Triglycerides (2-2-2 to 22-22-22) Used To Determine Retention Time/Acyl Carbon Number Matrix for This Reconstruction Method

triglyceride	ACN (mg)	concn (mg/mL)	RRF(X)[a]	ACN span	av RRF	triglyceride group
2-2-2	6	0.670	0.128	6−9	0.33	1
3-3-3	9	0.721	0.504			
(interpolated)				10−16	0.66	2
2-2-16	20	0.480	0.902	17−25	0.86	3
8-8-8	24	0.704	0.925			
9-9-9	27	0.716	0.955	26−33	0.98	4
10-10-10	30	0.484	1.002			
11-11-11	33	0.636	1.000			
12-12-12	36	0.584	1.040	34−41	1.02	5
13-13-13	39	0.532	1.054			
14-14-14	42	0.492	1.024	42−48	1.02	6
15-15-15	45	0.466	1.026			
16-16-16	48	0.508	1.020			
17-17-17	51	0.540	0.944	48−54	0.97	7
18-18-18	54	0.476	0.951			
19-19-19	57	0.480	0.798			
20-20-20	60	0.448	0.661			
21-21-21	63	0.476	0.539			
22-22-22	66	0.470	0.315			

[a] RRFs were calculated using (i) the definition of RRF given under Calculations; (ii) triplicate FID responses at three triglyceride standard concentrations [total concentration given above, half-concentration, and zero (blank) concentration]; and (iii) linear regressions of responses on mass of material injected. The slope of response versus mass of the given standard triglyceride divided by that of the internal standard (triundecanoin) gives the RRFs, combining the information content in the data and minimizing the error; the intercept terms in the regression equations were typically negligible.

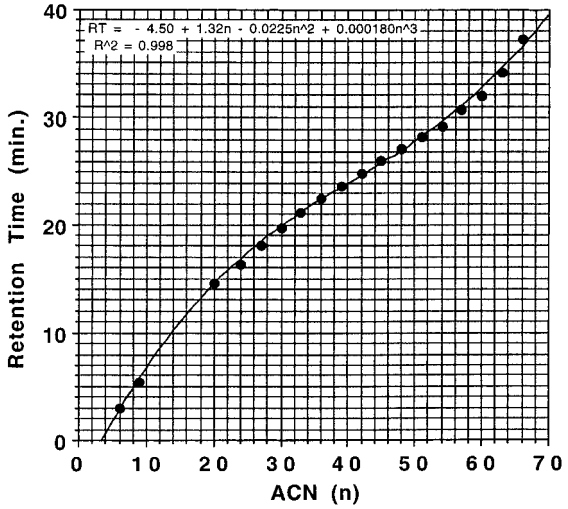

Figure 2. RT versus ACN for the mixture of predominantly homogeneous triglycerides shown in Figure 1. A third-order polynomial regression of RT on ACN is a fast and reproducible means to statistically estimate all intermediate RTs (within the temporal range) given ACNs.

injection of up to ~150 ng of given compounds (i.e., 0.5 μL of an ~0.35 mg/g solution) gives excellent linearities over 2−3 (and even higher) orders of magnitude in mass. In the 0−150 ng per injection mass range, we typically linearly calibrate with two data points (0 and 150 ng per injection), assuming linearity based on experience. The triundecanoin in the reference solution permits the calculation of the RRFs, as described below.]

(4) Combine 1.0 mL of each of all of the standard triglyceride solutions to produce a mixture from which to simultaneously determine the RTs of the standards. Analyze by GC (Figure 1). Typical RRFs of functionally related groups are given in Table 1.

Notes. (1) Determination of RRFs should be performed prior to the first analysis of triglyceride samples. Redetermination of RRFs should be undertaken at approximately monthly intervals or when significant changes to the GC-FID occur (e.g., replacement of GC column, cleaning of flame ionization detector etc.). (2) When RRFs are evaluated, percent relative standard deviation (% RSD) of the RRFs for each component should be <5%.

Definitions. RRFs. The RF of a chromatographable compound is defined as

$$\mathrm{RF}_x \equiv A_x/M_x \tag{1}$$

where RF_x is the response factor of compound x (kcts/ng; kcts = 10^3 counts), A_x is the area count of compound x (kcts), and M_x is the mass of compound x [ng; e.g., 0.5 μL·(200 ng/μL) = 100 ng].

Thus, the RRF is defined as the ratio of the RF of a given compound x relative to that of a standard compound (namely, t = triundecanoin):

$$\mathrm{RRF}_x \equiv \frac{\mathrm{RF}_x}{\mathrm{RF}_t} = \frac{A_x/M_x}{A_t/M_t} \tag{2}$$

Rearranged for ease of application, RRF is typically expressed as

$$\mathrm{RRF}_x = \frac{A_x M_t}{M_x A_t} \tag{3}$$

RESULTS AND DISCUSSION

Development and Application of the Statistical, ACN-Based Technique. *Statistical Determination of RTs and Chromatographic Groupings of Benefat S.* To determine the statistically estimated RTs (and thereby the ACN-delineated chromatographic groupings of Benefat S), the ACNs of the standard suite of homogeneous triglycerides (6−66) are regressed upon their RTs with a third-order polynomial [e.g., see Snedecor and Cochran (1978) or other computerized, higher-order regression methods; Figure 2]. In present applications, the correlation coefficients are typically very high (typically, $r^2 \geq 0.998$). With that regression [RT = f(ACN)], a column of estimated RTs is calculated from a column of integral ACNs (viz., 6−72; Table 2). Predetermined groups of triglycerides (based on the composition of their fatty acid side chains) from a limited suite of authentic standards indicate which ACNs will define limits of the groups (Table 3 and discussed further below). In the case of Benefat S, the ACN groups correspond to the following triglyceride compositions (*group number*, ACN span, group type): *1*, 6−9, SSS; *2*, 10−16, SSM; *3*, 17−25, SMM, SSL; *4*, 26−33, SML; *5*, 34−41, SLL, MML; *6*, 42−48, MLL; and, *7*, >49, LLL), where S = short-chain (C_{2-4}), M = medium-chain ($C_{6,8,10,12}$), and L = long-chain ($C_{16,18}$) triglycerides (Table 4).

Mass Percent of Given Triglyceride Groups. Rearrangement of eq 3 shows that the mass of a given compound (M_x) can be calculated from its peak area (A_x), mass of triundecanoin (M_t), the RRF_x, and A_x:

$$M_x = \frac{A_x M_t}{(\mathrm{RRF}_x)A_t} = \frac{A_x}{(\mathrm{RRF}_x)(\mathrm{RF}_t)} \tag{4}$$

Because (i) the mass percentage of a total chromatogram represented by given compound groups is the goal

Table 2. Calculated RTs and GC Group Limits

ACN	RT (estd)[a] (min)	limit (min)	group (*n*)
6	2.68		
7	3.72		
8	4.73		
9	5.71	6.18	1
10	6.65		
11	7.55		
12	8.43		
13	9.27		
14	10.08		
15	10.86		
16	11.61	11.97	2
17	12.34		
18	13.04		
19	13.71		
20	14.36		
21	14.98		
22	15.59		
23	16.17		
24	16.73		
25	17.28	17.54	3
26	17.80		
27	18.31		
28	18.80		
29	19.28		
30	19.75		
31	20.20		
32	20.64		
33	21.07	21.28	4
34	21.49		
35	21.91		
36	22.31		
37	22.71		
38	23.11		
39	23.50		
40	23.89		
41	24.27	24.47	5
42	24.67		
43	25.04		
44	25.43		
45	25.82		
46	26.21		
47	26.61		
48	27.01	27.22	6
49	27.42		
50	27.84		
51	28.27		
52	28.70		
53	29.15		
54	29.61		
55	30.08		
56	30.57		
57	31.07		
58	31.59		
59	32.12		
60	32.67		
61	33.25		
62	33.84		
63	34.46		
64	35.09		
65	35.76		
66	36.44		
67	37.15		
68	37.89		
69	38.66		
70	39.46		
71	40.28		
72	41.14	>49.0	7

[a] Calculated RTs are estimated by the statistical method discussed in text.

Table 3. Nabisco's Description of Benefat S

group	ACN (*n*)	triester strucutre:type
1	6	2-2-2:SSS
1	7	2-2-3:SSS
1	8	2-3-3:SSS
1	9	3-3-3:SSS
2	10	2-2-6:SSM
2	11	2-3-6:SSM
2	12	2-2-8:SSM
2	13	2-3-8:SSM
2	14	3-3-8:2-2-10:SSM
2	15	2-3-10:SSM
2	16	3-3-10:SSM
3	17	
3	18	2-8-8:SMM
3	19	3-8-8:SMM
3	20	2-8-10:2-2-16:SMM:SSL
3	21	3-8-10:2-2-16:SMM:SSL
3	22	2-10-10:2-2-18:SMM:SSL
3	23	2-3-18:SSL
3	24	3-3-18:8-8-8:SSL:MMM
3	25	2-3-20:SSL
4	26	2-8-16:SML
4	27	3-8-16:SML
4	28	2-8-18:SML
4	29	3-8-18:SML
4	30	2-10-18:10-10-10:SML:MMM
4	31	3-10-18:SML
4	32	2-10-20:SML
4	33	3-10-20:SML
5	34	8-8-18:MML
5	35	3-16-16:SLL
5	36	8-10-18:2-16-18:MMLSLL
5	37	3-16-18:SLL
5	38	2-18-18:8-10-20:10-10-18:SLL:MML:MML
5	39	3-18-18:SLL
5	40	2-18-20:SLL
5	41	3-18-20:SLL
6	42	8-16-18:MLL
6	43	
6	44	8-18-18:MLL
6	45	
6	46	10-18-18:MLL
6	47	
6	48	10-18-20:MLL
7	49	
7	50	16-16-18
7	51	
7	52	16-18-18:LLL
7	53	
7	54	18-18-18:LLL
7	55	
7	56	18-18-20:LLL
7	57	
7	58	18-20-20:LLL

of this calculation and (ii) RF_t is nearly constant (within tenths of a percent for all compounds analyzed under similar conditions), RF_t is taken to equal 1 and the masses (relative to other compounds in the chromatogram) can be calculated (see Table 5):

$$M_x \propto A_x/\mathrm{RRF}_x \quad (5)$$

(Of course, determination of RF_t will allow the absolute determination of the masses of the individual peaks in the chromatogram, but this step is unnecessary here.)

Because all of the individual compounds' standards of the complex mixture are not available where this technique is applied, RRFs of a given ACN group (e.g., 6−9) are averages of the RRFs of all of the available standard compounds in that ACN range (Figure 3 and Table 1). The RSDs associated with these approximations are straightforwardly calculated and typically range between 1 and 10%, giving a quantitative esti-

Table 4. Chromatographic Data of Benefat S[a]

peak	retention time (min)	area (kcts)	group (*n*)
1	0.945		
2	1.102	4.346	
3	1.655	6.336	1
4	10.649	2.042	
5	11.353	5.109	
6	11.493	2.618	2
7	12.668	6.429	
8	12.784	3.411	
9	13.284	7.797	
10	13.355	4.532	
11	14.065	10.568	
12	14.185	4.128	
13	14.537	19.936	
14	14.641	11.748	
15	14.718	5.864	
16	15.078	46.151	
17	15.420	28.881	
18	15.643	26.323	
19	15.899	177.201	
20	15.996	28.471	
21	16.426	389.839	
22	16.945	207.125	
23	17.479	6.876	3
24	17.851	11.443	
25	17.951	10.629	
26	18.322	12.842	
27	18.402	14.825	
28	18.791	11.925	
29	19.000	95.041	
30	19.095	44.436	
31	19.447	100.861	
32	19.510	47.358	
33	20.008	37.892	
34	20.119	19.043	
35	20.430	36.576	
36	20.506	18.324	4
37	21.193	860.982	IS[b]
38	21.554	3.081	
39	21.668	25.506	
40	21.930	2.696	
41	22.014	7.726	
42	22.307	2.841	
43	22.544	19.381	
44	22.856	39.156	
45	22.970	17.144	
46	23.215	37.102	
47	23.301	19.787	
48	23.383	4.818	
49	23.759	147.707	
50	23.862	62.435	
51	24.101	141.014	
52	24.182	68.771	5
53	25.038	23.889	
54	25.830	72.551	
55	26.518	27.291	6
56	27.872	4.871	
57	28.546	28.107	
58	29.201	68.161	7

[a] As reported by the GC computerized data system. [b] IS, internal standard, that is, triundecanoin.

mate of the error associated with the present statistical estimations of mass per group.

For perspective, the calculation of the composite RSD associated with the individual RSD (e.g., 1−5%/compound) for 90 compounds is shown in the Appendix. For a worst acceptable case of ±5% RSD per individual compound's RRF for each of the ~90 compounds, the composite RSD of the total mass would be ±47%—unacceptable for almost all applications. Even with only 1% RSD per compound, the total RSD would be ±9.5%—still quite high and marginally acceptable for most applications. Reducing the number of quantified groups from the sum of many individual compounds by the present ACN-based and functionally related grouping reduces the overall error by decreasing the number of individual errors that are propagated.

Table 5. Benefat S Mass Percent per Group

group	area	RRF	relative mass[a]	mass (%)
1	0	0.33	0	0.0
2	9.8	0.66	15	0.6
3	985.3	0.86	1146	46.9
4	461.2	0.98	471	19.3
5	599.2	1.02	587	24.0
6	123.7	1.02	121	5.0
7	101.1	0.97	104	4.3
total			2444	100.0

[a] As discussed following eq 5, the RF of the internal standard (11-11-11) is taken to be constant at one since absolute masses are not required for the present calculation.

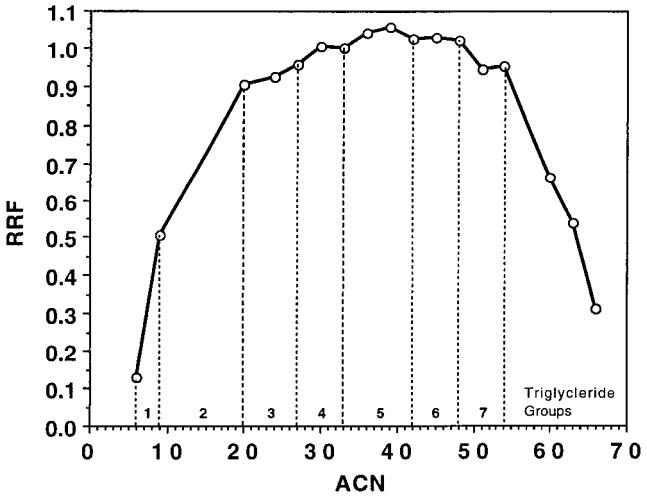

Figure 3. RRFs of the mixture of predominantly homogeneous triglycerides (2-2-2 to 22-22-22) plotted as a function of ACN.

Thus, the mass percent of compounds **1**, **2**, ..., ***n*** out of a total mass (M_{tot}) is calculated as

$$M\,(\%) = \frac{(M_1 + M_2 + \ldots + M_n)}{M_{tot}} \times 100\% \qquad (6)$$

where M_1, M_2, ..., M_n = masses of compound peaks **1**, **2**, ..., ***n*** in a given group.

Subdividing the Chromatogram into Groups. A gas chromatogram of Benefat S triglyceride prototype sample containing 58 integrated peaks is shown in Figure 4, and its chromatographic data are shown in Table 2. Previous analyses of numerous Salatrim mixtures have shown and straightforward statistical calculations indicate that identification of one or a few compounds (via standards) in a given group gives a very good indication of compound-group's identities (e.g., SSS, SSM, etc., in Figure 4). The partitioning of the seven functionally associated groups was based on the preceding knowledge of the functional groups' limits and the associated individual ACNs derived from the regression of RT on ACN. The strength of this method is that with these ACN-based partitions established in one laboratory, they are easily established in another. [For example, comparison of our seven-group partitioning with our toller's independent identifications of the individual

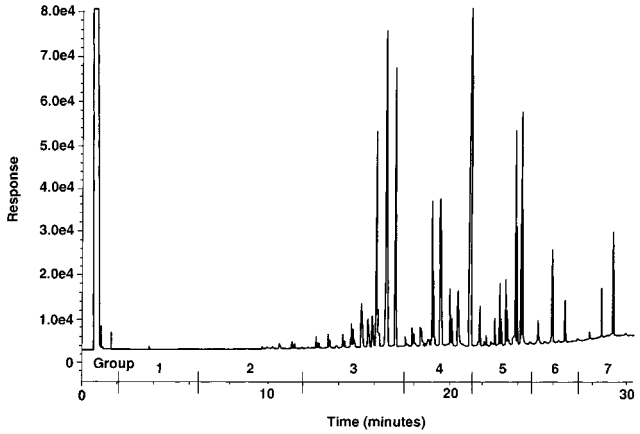

Figure 4. Subdivision of chromatogram of the Benefat S triglyceride sample into seven RT-based groups established by the present ACN-based method. The RT partitions are given in Table 2. The numerical groupings represent the following triglycerides (*group number*, ACN span, group type): *1*, 6−9, SSS; *2*, 10−16, SSM; *3*, 17−25, SMM, SSL; *4*, 26−33, SML; *5*, 34−41, SLL, MML; *6*, 42−48, MLL; *7*, >49, LLL), [S = short-chain (C_{2-4}), M = medium-chain ($C_{6,8,10,12}$), and L = long-chain ($C_{16,18}$) triglycerides].

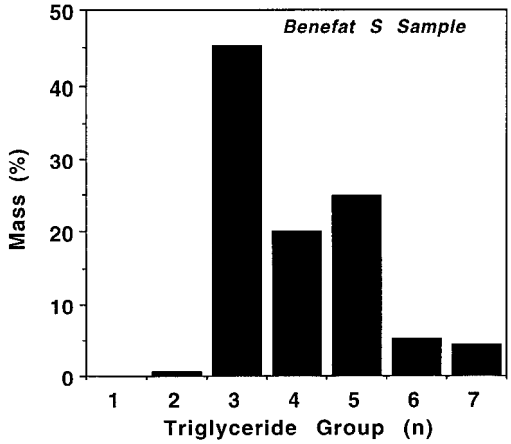

Figure 5. Histogram of the mass percent of each of the seven groups of triglycerides in a Benefat S sample mixture.

compounds of the prototype Benefat S sample gave an excellent mass-per-mass correlation ($r^2 = 1.000$) for the same structural groups.] Thus, this method can be considered an "ACN-meter" for partitioning triglycerides, analogous to the *n*-alkane-based Kovat's retention index.

Summary of the Statistical, ACN-Based Technique. (A) Determine triglyceride group partitions by ACN (viz., *1*, 6−9; *2*, 10−16; *3*, 17−25; *4*, 26−33; *5*, 34−41; *6*, 42−48; and, *7*, >49). (B) Calibrate ACN scale as a function of RT: (1) Determine RTs of a suite of homogeneous triglycerides spanning a broad range of ACNs 2-2-2 (ACN = 6) to 22-22-22 (66)] (Figure 1). Use homogeneous standards and fill in the low ACN gaps with nonhomogeneous triglycerides [e.g., 2-2-16 (20)]. (2) Plot RT (*Y*) versus ACN (*X*) and regress RT upon ACN using an appropriate regression equation (Figure 3). A third-order polynomial worked very well in this case. (3) In a computer spreadsheet, generate a column of ACNs from 6 to 72. Using the previous regression equation [RT = *f*(ACN], calculate the reconstructed RTs for all of the ACNs (Table 2). With that, a continuous suite of RTs (based on ACN data), the variance of which has been statistically minimized over a suite of compounds, is generated. (4) In the spreadsheet, calculate the RT midpoint between two triglyceride groups (e.g., between groups 1 and 2). Use those specific RTs to partition the Benefat S chromatograms into seven groups, as above (Tables 2, 4, and 5). The total peak area of each of the seven groups is then summed. A representative RRF (viz., RRF versus 11-11-11; Figure 2) for each of the groups shall be used to calculate the mass percent of each of the seven groups relative to the total mass (Figure 5).

CONCLUSIONS

At times it is not feasible to identify and quantify the composition of each of the unidentified compounds in complex mixtures. The method described here is a provisional means by which to reproducibly subdivide the compounds of complex chromatograms into ACN-spanning groups—and to a large extent, functional groups (e.g., SSS, SSM, etc.)—until all of the compounds can later be individually identified and quantified via authentic standards. A strength of this method is its ability to reproducibly subdivide complex chromatograms on different analytical instruments at different locations, thus allowing compositional comparisons. In particular, it was successfully developed to partition complex triglyceride mixtures into functional groups as a synthetic production method was scaled up from the kilogram to many-ton production facilities at great distances. Finally, this carbon-number-based method can be applied to other complex mixtures as well: carbon numbers based on any complex compound class can be calibrated into a carbon number scale and then used to subdivide and quantify complex mixtures and intercalibrate instruments and synthetic processes.

ACKNOWLEDGMENT

I gratefully acknowledge the encouragement of James Miller of Cultor Food Sciences, who has encouraged publication of this research. I thank Maha Khaled, Lawrence Klemann, Ping Chang, and Edward Watkins for helpful comments on the manuscript and Grace Ordonio-Haas for development of the comprehensive gas chromatographic method used here.

APPENDIX: TOTAL ERROR AS A FUNCTION OF NUMBER OF SAMPLES AND INDIVIDUAL PERCENT ERROR

The summation of individual compounds to compose useful subgroups is potentially fraught with the problem of large accumulated errors. To examine the cumulative effect of the number of samples and individual percentage errors on the total error of a system, the following analysis of error is performed. A practical example is the composition of useful subgroups from a complex mixture such as Benefat S that may be composed of ~90 individual compounds. Quantitation of the total error as a function of the total number of compounds (viz., 1−90) and the individual percentage error (1−5%) can be calculated as a sum of the squares of the individual errors

$$\epsilon_t = [n(\epsilon_i)^2]^{0.5}$$

where ϵ_t is the total error as a fraction, n is the total number of measurements, and ϵ_i is the fractional error of individual measurements.

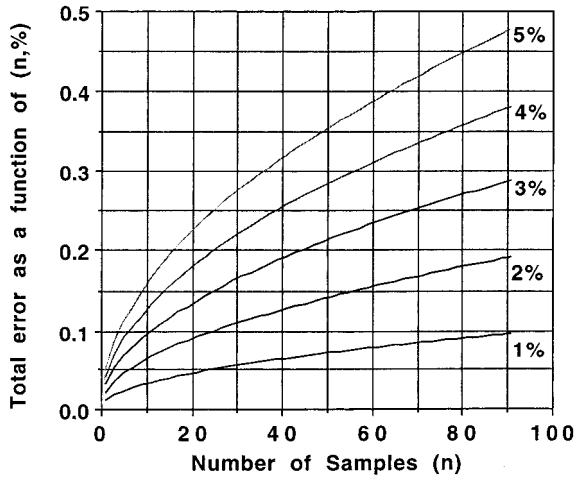

Figure 6. Total error plotted as a function of sample (compound) number and percentage error per sample as a function of number of samples (*n*).

The results are shown graphically in Figure 6. The total errors rapidly mount as the total of 90 measurements is approached, with as little as 9.5% total error (given 1% individual error per compound) to as much as 47% total error (given 5% error per compound). These results indicate that care must be taken in the evaluation of the compounded errors of numerous measurements. They also suggest that one should consider the point of diminishing return in the selection of an analytical method.

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Received for review January 22, 1999. Revised manuscript received December 21, 1999. Accepted January 5, 2000.

JF990059T