Determination of D003 by capillary gas chromatography

David Marrero Delange,* Ernesto Méndez Antolín, Víctor González Canavaciolo, Miriam O. Cora Medina and Abilio Laguna Granja

Center of Natural Products, National Center for Scientific Research (CNIC), P.O. Box 6414, Cubanacán, Playa, Havana City, Cuba.
Fax:(537) 336837
E-mail: david_delange@yahoo.com or dalmer@ip.etecsa.cu

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RESUMEN: El D003 es una mezcla de ácidos grasos saturados de cadena muy larga en un intervalo de 24 a 36 átomos de carbono, obtenido de la cera de caña de azúcar (*Saccharum officinarum* L.), el cual presenta efectos antiplaquetarios, antitrombóticos, antioxidantes y como reductor del colesterol. Se desarrolló y validó un método para la determinación de este producto mediante la cromatografía gaseosa capilar. Los ácidos fueron analizados como ésteres metílicos y separados en una columna wide-bore BP-5, empleando el ácido 1-nonacosanoico como patrón interno. Se encontró buena linealidad (r > 0,99; CVs de los factores de respuestas y de las pendientes fueron menores que 5 y 2 %, respectivamente); así como ausencia de sesgo, tanto para los ácidos individuales como para el total en todo el intervalo de concentración estudiado, desde 30 a 150 % de la masa nominal. También se obtuvieron recobrados cuantitativos (98,9 y 100,62%) y una buena precisión intermedia (CV = 0,96 %). El estudio de especificidad no mostró interferencias respecto a la determinación de esta mezcla, una vez que las muestras fueron sometidas a condiciones de estrés. Conforme a estos resultados, se puede asegurar que el procedimiento validado es apropiado para el control de la calidad y los estudios de estabilidad de este producto.

ABSTRACT: D003 is a mixture of saturated very long-chain fatty acids ranging from 24 to 36 carbon atoms, obtained from sugar cane (*Saccharum officinarum* L.) wax, with antiplatelet, antithrombotic, antioxidant and cholesterol-lowering effects. A capillary gas chromatographic method for the determination of this product was developed and validated. The acids were analyzed as methyl ester derivatives and separated in a BP-5 wide-bore column using 1-nonacosanoic acid as internal standard. A good linearity (r > 0.99; CVs of the response factors and of the slopes lower than 5 and 2 %, respectively); as well as absence of bias was found for both, total and individual acids in the whole studied concentration range, from 30 to 150 % of the nominal mass. Quantitative recoveries (98.9 and 100.62 %) and good intermediate precision (CV = 0.96 %) were also obtained. The specificity study showed no interferences regarding the determination of this mixture, once the samples were submitted to stress conditions. According to these results, it can be ensure that the validated procedure is appropriate for the quality control and stability studies of this product.

INTRODUCTION

Fatty acids (FAs) are important constituents of waxes, in which they occur in free and esterified forms. These compounds are found in animal, plant and microbial tissues and have a variety of functions, such as energy stores and waterproofing. [1,2] D003 comprises a natural mixture of higher primary aliphatic acids ($C_{24:0}$ to $C_{36:0}$) obtained from sugar cane (*Saccharum officinarum* L.) wax.[3] This product was found to be responsible for some biological properties related to health, such as antioxidant [4] and cholesterol-lowering effects, determined both *in vivo* and *in vitro* models,[5,6] as well as antiplatelet and antithrombotic activities.[7] Previous acute and subchronic studies of the oral toxicity of D003, conducted in rodents, have shown that doses up to 5 g/kg (acute study) and 1.250 mg/kg (subchronic study) do not induce any product-related toxicity.[8] It was also found that this mixture does not show evidences of cytotoxic or genotoxic activity on both somatic or germ cells in rodents.[9] The FAs are usually converted to methyl esters derivatives (FAMEs) and analyzed by Gas Chromatography (GC).[10,11] However, the reported data for the quantitative analysis of those acids larger than 26 carbon atoms, lack of enough accuracy and precision.

In light of these findings, a method for determining individual and total fatty acids content of D003 is developed and validated following well-established criteria.[12,13]

MATERIALS AND METHODS

Chemicals

D003 (batch 990702) was provided by CNIC (Havana, Cuba); all other chemicals were analytical reagent grade: Hydrochloric acid HCl (37 %), Methanol CH_3OH, Toluene, HCl (0.1 mol/L), hydrogen peroxide (H_2O_2, 30 %), NaOH (0.1 mol/L) (Merck, Darmstadt, Germany), and Chloroform $CHCl_3$ (Riedel-de-Haën, Seelze, Germany).

Internal standard (IS) solution: 1mg/mL, 1-nonadecanoic acid ($C_{19:0}$, Sigma,USA) in $CHCl_3$. Stock solution, 6.6 mg 1-tetracosanoic ($C_{24:0}$), 4,6 mg 1-pentacosanoic ($C_{25:0}$), 13,9 mg 1-hexacosanoic ($C_{26:0}$), 12 mg 1-heptacosanoic ($C_{27:0}$), 150.1 mg 1-octacosanoic ($C_{28:0}$), 8 mg 1-nonacosanoic ($C_{29:0}$), 80 mg 1-triacontanoic ($C_{30:0}$) and 5 mg 1-hentriacontanoic ($C_{31:0}$) acids; all > 99 % GC, (Sigma, St. Louis, MO, USA or equivalent) into a 100 mL volumetric flask to give final concentrations of 0.07, 0.05, 0.14, 0.12, 1.50, 0.08, 0.8, and 0.05mg/mL^{-1},respectively. Complete the volume with $CHCl_3$ and mix.

To prepare the working standard solution, accurately weigh 305 mg D003 in a 250 ml volumetric flask of $HCCl_3$. Dilute to volume with $CHCl_3$ and mix.

All these solutions were found to be stable for at least 1 month when stored at +4 °C.

To prepare the methylating solution (MSoln), mix 5 mL HCl and 95 mL methanol into a 100 mL volumetric flask. This solution should be weekly prepared and stored at +4 °C.

Equipments

Laboratory 1. A Shimadzu GC 14A equipment fitted with a wide bore adapter in the injection port intended for packed column and a flame-ionization detector (FID) was used. Individual FAME was separated with a 30 m x 0.53 mm fused silica column coated with 1.0 µm DB-5 (J&B Scientific, Folsom, USA). Peak areas were processed with a Shimadzu CR4A computing integrator. Samples (1 µL) were injected at a column temperature of 250 °C by the "solvent flush" technique;[14] then, the temperature was raised at 5 °C/min to 320 °C, with a final time of 10 min. The injector and detector were set at 300 and 320 °C respectively. Carrier gas flow (H_2) was 11.4 mL/min.

Laboratory 2. It was only used in the intermediate precision study. A Shimadzu GC 14B chromatograph was employed as previously described, but it was used an SGE (Austin, Tx, USA) BPX-5 wide bore column (25 m x 0.53 mm I.D, 1.0 µm film thickness). Oven temperature was programmed from 220 to 340 °C at 5 °C/min and held for 10 min at the final temperature. Injector and detector temperatures were 320 °C and 340 °C respectively. Argon was used as carrier gas at 4.5 mL/min.

Peak identification of acids that occur in D003 was performed by gas chromatography-mass spectrometry (GC-MS) analysis. It was used a gas chromatograph GC 8000 (Fisons Instruments, Italy), equipped with a mass selective detector MD800 on-line coupled to Lab-base software (VG, Masslab, UK). An SPB-5 fused silica capillary column (30 m x 0.25 mm I.D., 0.5 µm film thickness) from Supelco (Bellfonte, PA, USA) was used for individual separation of the compounds. GC conditions: Injector temperature, 300 °C; oven temperature gradient, from 100 to 200 °C at 40 °C /min, then increased by 10 °C /min from 200 to 320 °C and subsequently kept at 320 °C for 30 min. Injector parameters: split-splitless mode, septum purge flow-rate 5 mL/min and a split-vent flow-rate at 45 mL/min, and closed for 60 s. 1 µL portions were injected. Ion source and interface temperature were 250 °C and 250 °C, respectively. Ionization energy was 70 eV. The mass spectrum was continuously acquired from 40 to 600 m/z with a scan speed of 1 sec/decade in full scan mode. The carrier gas (Helium) flow was 1 mL/min.

Sample preparation

Samples were processed as described by González et al.[15] Briefly, 10 mg test material, was accurately weighed in a 1.8 mL vial and 1 mL of $C_{19:0}$ IS solution was added, then the solvent was evaporated to dryness at 80 °C under a gently nitrogen flow. It was removed and cooled to room temperature. One mL of freshly made MSoln was added; afterwards, the vial was tightly capped and placed into a block heater at 80°C for 90 min, with occasional shaking. After cooling, the vial was opened and the sample was evaporated to dryness. Then, 1 mL of toluene was added to the dry methyl ester mixture and the vial was once again tightly closed and heated at 80 °C for 3 min, this way the sample was ready to GC analysis (1µL injection volume).

GC separation and quantitation of FAs.

GC identification of individual FAMEs according to their relative retention ($r_{i,s}$), as well as their quantitation, were based on the internal standard method.[16]

The composition (%) was calculated according to the following equation:

$$C_i = \frac{A_i \cdot f_i^m \cdot m_{is} \cdot 100\,\%}{A_{is} \cdot m_m}$$

where: C_i = Content of component i (%), A_i = peak area of the component I, m_{is} = mass of internal standard (mg), A_{is} = peak area of the internal standard, f_i^m = relative mass response factor, m_m = sample weight (mg).

For determining f_i^m there were taken 0.5 mL of the stock solution and 0.25 mL of the IS solution which were transferred to an 1.8 mL crimp vial, the content was evaporated to dryness at 80 °C with nitrogen stream. Afterwards, the methylation process was carried out as described above; and after drying, 250 µL of toluene were added. The ester mixture was heated at 80 °C for 3 min, and 1 µL of this solution was analyzed. This procedure was

performed in triplicate, and f_i^m was calculated as follows:

$$f_i^m = \frac{A_{is} \cdot m_i}{A_i \cdot m_{is}}$$

Because of commercial standard of 1-dotriacontanoic ($C_{32:0}$), 1-tritriacontanoic ($C_{33:0}$), 1-tetratriacontanoic ($C_{34:0}$), 1-pentatriacontanoic ($C_{35:0}$), and 1-hexatriacontanoic ($C_{36:0}$) acids were unavailable, the f_i^m of $C_{30:0}$ was used for the quantitation of the even acids and the f_i^m of $C_{31:0}$ for the odd ones. The total content of acids in D003 was determined as the sum of each acid percentage.

Validation of Test Procedure

Specificity

By comparing the chromatograms of the IS solution, the working standard solution of D003 and the D003 stressed under degradation conditions it was proven the specificity of the chromatographic system. For promoting the formation of degradation products, the D003 was subjected to thermolysis (105 °C, 2 weeks), base and acid hydrolysis (sample suspended in NaOH 0.1 mol/L and HCl 0.1 mol/L, respectively, at 1 g in 10 mL, at 105 °C, 1 day), oxidation (sample suspended in 30 % H_2O_2, at 1 g in 10 mL, at 25 °C, 1 week), and photolysis (254 nm UV light, at 25 °C, 1 week). These assays were carried out in neutral glass ampoules, which were previously flushed with nitrogen before sealed (n = 3). Peak purity was checked by GC-MS analysis.

System linearity

The linear dynamic range of the system was evaluated in five mass relationships (acid mass/IS mass) analyzed in triplicate. For this purpose 0.5, 1, 2, 3, and 4 mL of the stock solution were taken and 1 mL of the IS solution was added to each one. Afterwards, samples were evaporated to dryness, and analyzed according to the above-described procedure.

The regression lines (y = a + bx) were calculated by the method of least squares based on the chromatographic response ratios ($y = A_i/A_{is}$) versus the injected mass ratios ($x = m_i/m_{is}$). Evaluation was made by linearity and proportionality tests for p = 0.05, taking into account the following acceptance criteria:

- Correlation coefficient (r) ≥ 0.99.
- Relative standard deviation of response factor (RSD_f) < 5 %, where response factor is defined as y/x.
- Relative standard deviation of slope (RSD_b) ≤ 2 %, with

$$RSD_b(\%) = \frac{SD_b}{b} \cdot 100$$

Where:

SD_b = standard deviation of the slope.

- $t_{exp}b < t_{tab}(0.05, n-2)$, with

$$t_{exp}b = \frac{|b|}{SD_b}$$

- The zero value should be included in the confidence intervals (CI) of the intercept:

$$CI = a \pm t \cdot SD_a$$

Where:

SD_a = Standard deviation of the intercept.

Linearity of the method

The linearity was assessed at five concentration levels, from 30 to 150 % of the nominal concentration (n = 3). The following volumes: 0.5, 1, 1.5, 2.0, and 2.5 mL were taken from the working standard solution and transferred to the vials. Afterwards, 0.2 mL of the IS solution were added to each one and they were evaporated to dryness. Finally, the mixture was analyzed according to the analytical method, but adding 0.2 mL of toluene to the dry methyl ester mixture.

The regression lines were obtained as described in the system linearity study; but in this case, the calculated masses of the product (y) versus the analyzed masses (x) of the raw material were evaluated. Linearity and proportionality tests were done as indicated above.

Accuracy

Accuracy was assessed by a recovery study over the range 80-120 % of the nominal concentration (n = 3). The previously analyzed matrix (5.0 mL of working standard solution) was spiked with 0.4, 0.8, and 1.2 mL of the stock solution, and 1 mL of IS solution was added. Samples were evaporated to dryness, and then the mixtures were analyzed as previously described in sample preparation.

The mass (mg) of each acid was obtained from the equation used in the quantitative determination, but without using m_m and 100 %. Recoveries were calculated according to the following equation:

$$\text{Recovery}(\%) = \frac{\text{Amount found}}{\text{Amount added}} \cdot 100$$

Average recovery was checked to 100 % with the student t test. The experimental value of t was calculated as follows:

$$t = \frac{100 - \text{Recovery}}{RSD} \cdot \sqrt{n}$$

The null hypothesis (the recovery is closed to 100 % and the method is accurate) was accepted for a significance level greater than 5 %. In order to determine if the concentration factor affects the results, the Cochran test for p = 0.05 was employed.

Precision

Repeatability and intermediate precisions were evaluated by assaying a sample in two separate laboratories. Each operator followed the procedure under conditions of repeatability (n = 8). Fisher (F) and Student (t) tests for p = 0.05 were performed to determine significant differences between results. The RSD values were evaluated by comparison with the Horwitz's criterion.[17,18]

RESULTS AND DISCUSSION

GC-MS demonstrated that saturated FAs with chain lengths from C_{24} to C_{36} compose D003. Characteristic fragments of the FAMEs were examined and interpreted (mostly, m/z 74, 87, 143 and M^+) and the data were also compared with that observed in mass spectral library. Relative retentions of these FAMEs were used for GC identification (Table 1).

Figure 1 shows a superposition of chromatographic profiles corresponding to the acids that compose D003 (A) and the IS (B). It is quite evident that those compounds were well baseline separated. No significant interfering peak was observed even when samples were submitted to stress conditions, which shows the good selectivity of the proposed method.

System's linearity

Results of linearity and proportionality tests for regression curves (Table 2), demonstrate a linear response for all acids over the entire studied range, from 25 to 200 % of the nominal mass relationship that will be evaluated by the method. Coefficients of regression were greater than the acceptation limit (0.99), this means that the correlations are positives with a probability higher than 99.0 %. It can also be noted that the RSD_f and RSD_b were both bellow than acceptance criteria, 5 and 2% respectively.

From this table could also be inferred that the $t_{exp}b$ of the slope for every acid and for the total were greater than the t_{tab}, denoting that there is a probability > 95 % that the slopes been different to zero value. The confidence intervals of the intercepts included the zero value, which indicate that all regression lines passed through the origin. It was confirmed by experimental t values, which were lower than t_{tab} for all cases, therefore the system do not present bias. Taking into account these results, it can be said that the instrumental system is linear and proportional in the studied range.

Method's linearity

Linear relationships were found between the calculated and the added masses (Table 3). The coefficients of regression for all regression lines were greater than the acceptation limit (0.99), indicating that correlations are positives with a probability higher than 99.0 %. It can be appreciated that RSD_f were generally lower than 5 %, with the exception of $C_{35:0}$ because of its minority character in the mixture, and the RSD_b were all bellow than the acceptance criteria (2 %).

From this table it can also be observed that the $t_{exp}b$ of the slope for each acid and for the total was greater than the t_{tab}, meaning that there is a probability > 95 % that the slopes been different to zero value. Regarding the CI of the intercepts, it can be ensure that all of them included the zero value, which denote that the null hypothesis is fulfilled and there is a probability > 95 % that the intercepts be zero. In such a way, the intercept's test t gave $t_{exp}a < t_{tab}$ for all cases; therefore, no bias was found for the method. Considering these results, it may be affirmed that the method is linear and proportional over the whole concentration range.

Accuracy

It is generally assumed that long chain FAs (>12 carbon atoms) separated by GC produce an FID response that is directly proportional to the injected mass.[11] It has also been demonstrated that this response does not differ from unity to a great extent for the tested FAs, irrespective of chain length.[19] These simple assumptions hold true for those acids composing D003, according to the calculated f_i^m, which ranged between 0.93 and 0.99 with RSDs < 1 %.

In the present work, the mean recoveries from spiked samples (Table 4) were all between 98.9 and 100.62 % with good precision (RSD = 0.5 –1.2 %). The G_{exp} (0.417) was < G_{tab} (0.5612), therefore the concentration factor did not affect the variability of results. Recoveries and 100 % value were not significantly different, either for each concentration or for the total average recovery, according to the t_{exp} values, which were lower than tabulated t for p = 0.05 (4.303 and 2.306, respectively); consequently, the method is accurate.

Precision

Repeatability was good for both series of analysis (Table 5), according to within-day RSD values, which were lower than the calculated by Horwitz (2 %).[17,18] For minoritary acids there were obtained higher RSD, as expected, but total determinations were not affected.

No significant differences were found in either the precision or the means obtained from the two laboratories, according to the experimental F and t values (1.194 and 0.92, respectively), which were lower than the critical values for p = 0.05 (3.79 and 2.145, respectively). Total RSDs, regarding both series, were also lower than the acceptation criteria, these results prove that the method has a good intermediate precision. It can also be noted that the confidence limits for this batch were 76.59 ± 1.11 %; therefore in 95 of 100 cases, the analytical results will lie within the range 75.48 to 77.7 %.

CONCLUSIONS

It was validated a selective, precise and accurate gas chromatographic method for determination of acids that compose D003. The method was found to be useful for the quality control and stability studies of this product.

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Table 1
Average of GC relative retention for each acid of D003 (n = 3)

	$r_{i,s} \pm t \times SD/n^{1/2}$
$C_{19:0}$	IS
$C_{24:0}$	2.37 ± 0.02
$C_{25:0}$	2.73 ± 0.02
$C_{26:0}$	3.12 ± 0.02
$C_{27:0}$	3.51 ± 0.02
$C_{28:0}$	3.93 ± 0.02
$C_{29:0}$	4.33 ± 0.02
$C_{30:0}$	4.76 ± 0.02
$C_{31:0}$	5.16 ± 0.02
$C_{32:0}$	5.57 ± 0.02
$C_{33:0}$	5.99 ± 0.02
$C_{34:0}$	6.51 ± 0.02
$C_{35:0}$	7.14 ± 0.02
$C_{36:0}$	7.90 ± 0.02

Table 2
System's linearity data (t_{tab}= 2.160)

Acid	Linearity Test			Proportionality test			Correlation Coefficient (r)
	RSD_f (%)	$b \pm t \times SD_b$	RSD_b (rel)	$t_{exp}b$	$a \pm t \times SD_a$	$t_{exp}a$	
$C_{24:0}$	2.30	1.010 ± 0.021	0.97	103.33	0.001 ± 0.003	0.870	0.9994
$C_{25:0}$	2.25	1.031 ± 0.022	0.97	103.37	0.001 ± 0.002	0.200	0.9995
$C_{26:0}$	2.70	1.048 ± 0.026	1.14	87.40	0.004 ± 0.009	1.039	0.9993
$C_{27:0}$	1.97	1.029 ± 0.016	0.73	136.75	0.001 ± 0.005	0.347	0.9997
$C_{28:0}$	2.46	1.065 ± 0.025	1.07	93.13	0.053 ± 0.091	1.257	0.9993
$C_{29:0}$	2.55	1.053 ± 0.021	0.91	110.33	0.001 ± 0.004	0.115	0.9995
$C_{30:0}$	2.70	1.052 ± 0.023	1.01	99.49	0.034 ± 0.045	1.659	0.9994
$C_{31:0}$	2.80	1.027 ± 0.028	1.29	77.76	0.001 ± 0.004	0.310	0.9901
Total	2.54	1.049 ± 0.021	0.93	108.10	0.094 ± 0.145	1.399	0.9994

Table 3
Method's linearity data from GC determinations of D003 (t_{tab}= 2.160)

Acids	Linearity Test			Proportionality test			Correlation coefficient (r)
	RSD_f (%)	$b \pm t \times SD_b$	RSD_b (rel)	$t_{exp}b$	$a \pm t \times SD_a$	$t_{exp}a$	
$C_{24:0}$	1.91	0.012 ± 0.001	0.62	160.14	0.001 ± 0.002	1.418	0.9998
$C_{25:0}$	3.05	0.009 ± 0.001	1.14	88.12	0.002 ± 0.003	1.941	0.9993
$C_{26:0}$	1.73	0.025 ± 0.001	0.71	141.86	0.003 ± 0.004	1.699	0.9997
$C_{27:0}$	2.52	0.022 ± 0.001	1.28	78.25	0.004 ± 0.006	1.485	0.9992
$C_{28:0}$	1.96	0.285 ± 0.005	0.84	119.22	0.051 ± 0.052	2.096	0.9996
$C_{29:0}$	4.47	0.014 ± 0.001	1.95	51.29	0.005 ± 0.006	1.732	0.9981
$C_{30:0}$	2.57	0.157 ± 0.005	1.52	65.80	0.040 ± 0.052	1.651	0.9988
$C_{31:0}$	4.43	0.009 ± 0.001	1.93	51.85	0.002 ± 0.004	1.254	0.9981
$C_{32:0}$	2.42	0.082 ± 0.002	1.09	91.82	0.008 ± 0.019	0.929	0.9994
$C_{33:0}$	2.87	0.011 ± 0.002	1.40	71.22	0.003 ± 0.003	1.789	0.9990
$C_{34:0}$	3.20	0.097 ± 0.002	1.04	95.88	0.001 ± 0.022	0.130	0.9994
$C_{35:0}$	4.10	0.005 ± 0.000	1.26	79.34	0.001 ± 0.002	1.883	0.9992
$C_{36:0}$	3.89	0.034 ± 0.001	0.99	100.97	0.003 ± 0.007	1.010	0.9995
Total	2.15	0.767 ± 0.015	0.88	113.19	0.098 ± 0.148	1.425	0.9996

Table 4
Accuracy of the method (n = 3)

Amount added (mg)	Amount found (mg)			Mean recovery $\pm t \times SD/n^{1/2}$ (%)	RSD (%)	t_{exp}
	1	2	3			
1.120	1.111	1.122	1.117	99.70 ± 1.22	0.49	1.060
2.243	2.257	2.225	2.250	100.04 ± 1.85	0.75	0.092
3.363	3.375	3.358	3.326	99.70 ± 1.84	0.74	0.702
Total				99.82 ± 0.68	0.61	0.885

Table 5
Results of precision study between two laboratories (n = 8)

Acid	Laboratory 1 Mean (%) ± t x SD	RSD (%)	Laboratory 2 Mean (%) ± t x SD	RSD (%)	Both series Mean (%) ± t x SD	RSD (%)
$C_{24:0}$	1.24 ± 0.04	1.37	1.20 ± 0.03	1.17	1.22 ± 0.04	1.99
$C_{25:0}$	0.92 ± 0.05	2.28	0.89 ± 0.03	1.46	0.91 ± 0.03	2.52
$C_{26:0}$	2.53 ± 0.04	0.63	2.47 ± 0.06	1.01	2.50 ± 0.06	1.52
$C_{27:0}$	2.24 ± 0.08	1.65	2.27 ± 0.08	1.54	2.25 ± 0.06	1.67
$C_{28:0}$	28.59± 0.65	0.99	28.42 ± 0.62	0.95	28.51 ± 0.42	0.98
$C_{29:0}$	1.52 ± 0.05	1.45	1.47 ± 0.05	1.56	1.49 ± 0.03	2.12
$C_{30:0}$	15.94 ± 0.38	1.03	15.91 ± 0.36	0.99	15.93 ± 0.25	0.98
$C_{31:0}$	1.00 ± 0.03	1.50	1.01 ± 0.07	2.97	1.00 ± 0.02	2.38
$C_{32:0}$	8.21 ± 0.24	1.29	8.08 ± 0.18	1.00	8.14 ± 0.16	1.41
$C_{33:0}$	1.24 ± 0.11	3.94	1.19 ± 0.08	2.86	1.22 ± 0.07	4.02
$C_{34:0}$	9.54 ± 0.29	1.32	9.59 ± 0.26	1.19	9.56 ± 0.19	1.25
$C_{35:0}$	0.51 ± 0.04	3.82	0.52 ± 0.12	9.62	0.51 ± 0.03	7.17
$C_{36:0}$	3.28 ± 0.14	1.92	3.41 ± 0.13	1.64	3.35 ± 0.10	2.57
Total	76.76 ± 1.78	1.00	76.42 ± 1.63	0.92	76.59 ± 1.11	0.96

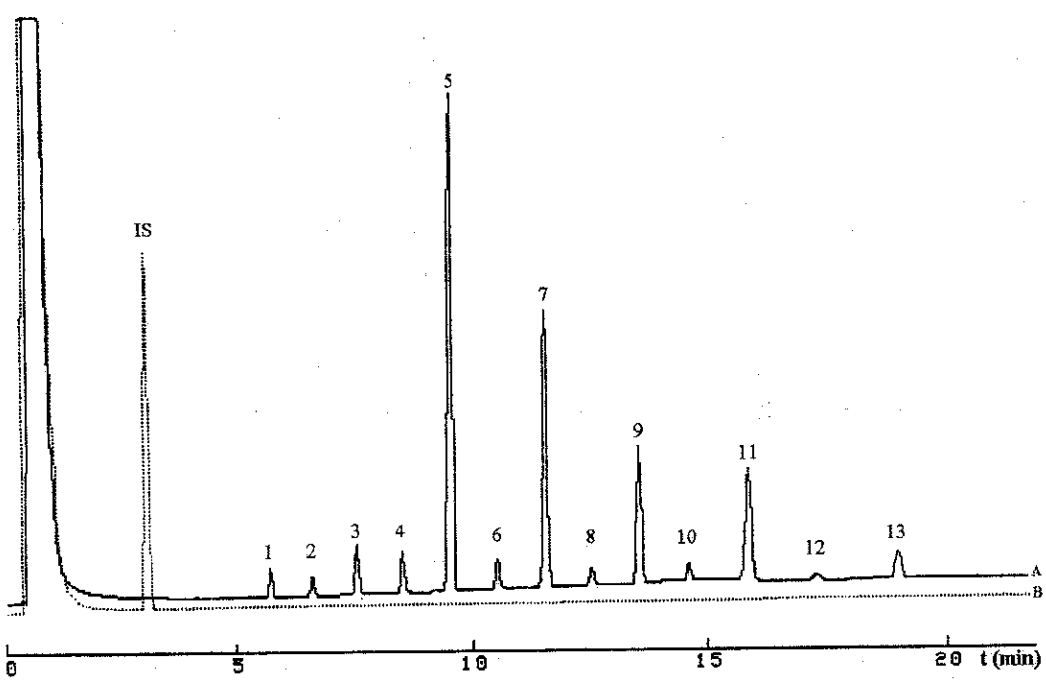

Figure 1: GC profiles of (A) FAs that compose D003 and (B) the internal standard, analyzed as methyl esters derivatives. Peaks: I.S ($C_{19:0}$), (1) $C_{24:0}$, (2) $C_{25:0}$, (3) $C_{26:0}$, (4) $C_{27:0}$, (5) $C_{28:0}$, (6) $C_{29:0}$, (7) $C_{30:0}$, (8) $C_{31:0}$, (9) $C_{32:0}$, (10) $C_{33:0}$, (11) $C_{34:0}$, (12) $C_{35:0}$, and (13) $C_{36:0}$.