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Reliable Identification and Quantification of Volatile Components of Sage Essential Oil Using Ultra HRGC

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Salvia officinalis (common sage) essential oil was chemically characterized by means of GC-MS, using 100 meter capillary columns with two different stationary phases. Identification of the analytes was carried out by means of the FFNSC mass spectrum library and the use of the Linear Retention Index (LRI) as an extra criterion of library searching. Quantitative analysis was also carried out by means of GC-FID with the internal standard method and the use of relative response factors determined for each chemical class of components. A total of 45 compounds were identified and quantified and reported with their experimental LRI values.

Keywords: sage oil, *Salvia officinalis* L., HRGC-MS, LRI, ultra HRGC, response factors.

Essential oils are complex matrices, mainly composed by terpenoids. A reliable identification, in the case of these compounds is a challenge, due to the very similar fragmentation pattern that these analytes produce when analyzed with a mass spectrometer detector. The simple comparison of the spectra acquired with those reported in the mass spectra databases can be a difficult issue driving to a wrong identification. A more reliable identification can be obtained using GC-MS information interactively with linear retention indices [1].

The complexity of these matrices has led to the use of more sophisticated techniques, such as comprehensive GC (GC×GC) and MDGC for their determination. However, these techniques require expensive instrumentation and specialized operators. In the present work, qualitative/quantitative characterization of the chemical composition of a sage essential oil, obtained by hydrodistillation (HD), were carried out by means of ultra HRGC-MS and HRGC-FID equipped with capillary columns (100 m in length), coated with both silphenylene polymer and polyethylene glycol, and results compared with those obtained by using columns of 30 m with the same stationary phases.

Salvia officinalis L. (Lamiaceae), commonly referred to as sage, has a long history of use as an aromatic and medicinal herb. Sages are cultivated in many countries and grow in the wild in the Mediterranean area. The sage essential oil is obtained from the aerial parts of the plants. The composition of the oil varies widely, depending on several factors, such as stages of development, climate and also between oil obtained from the vegetative and the floral part of the plants [2-4]. The main components of the essential oil of sage are reported in several works [5-8].

The commercial value of the essential oil is variable because it depends on the content of α-thujone and β-thujone. Thujones are known to cause permanent damage of the central nervous system and dementia at chronic intake. The European Committee sets maximum levels for thujone in foodstuffs and beverages at 0.5mg/kg due to the toxicity of these ketones while, regarding medicinal uses, ISO 9909 regulates the amounts of 11 components in the oil [9].

Analytes were identified using the laboratory constructed library dedicated to flavor and fragrance natural and synthetic compounds, FFNSC 1.3 (Shimadzu, Kyoto, Japan). This library contains for each compound the Linear Retention Index, in order to facilitate the identification of the analytes [10-11]. Data were processed through the GCMSsolution (Shimadzu) software.

The Retention Index system was proposed in 1958 by Kováts and it is based on the correlation between the retention time of the analytes and the ones of a series of references standards [12]. Reference standards used were *n*-alkanes and the retention index values were conventionally assigned by multiplying the carbon numbers for 100. On the basis of the Kovats equation, the Retention Index is defined as a number equal to 100 times the carbon number of a hypothetical *n*-alkane having the same retention time of the analytes.

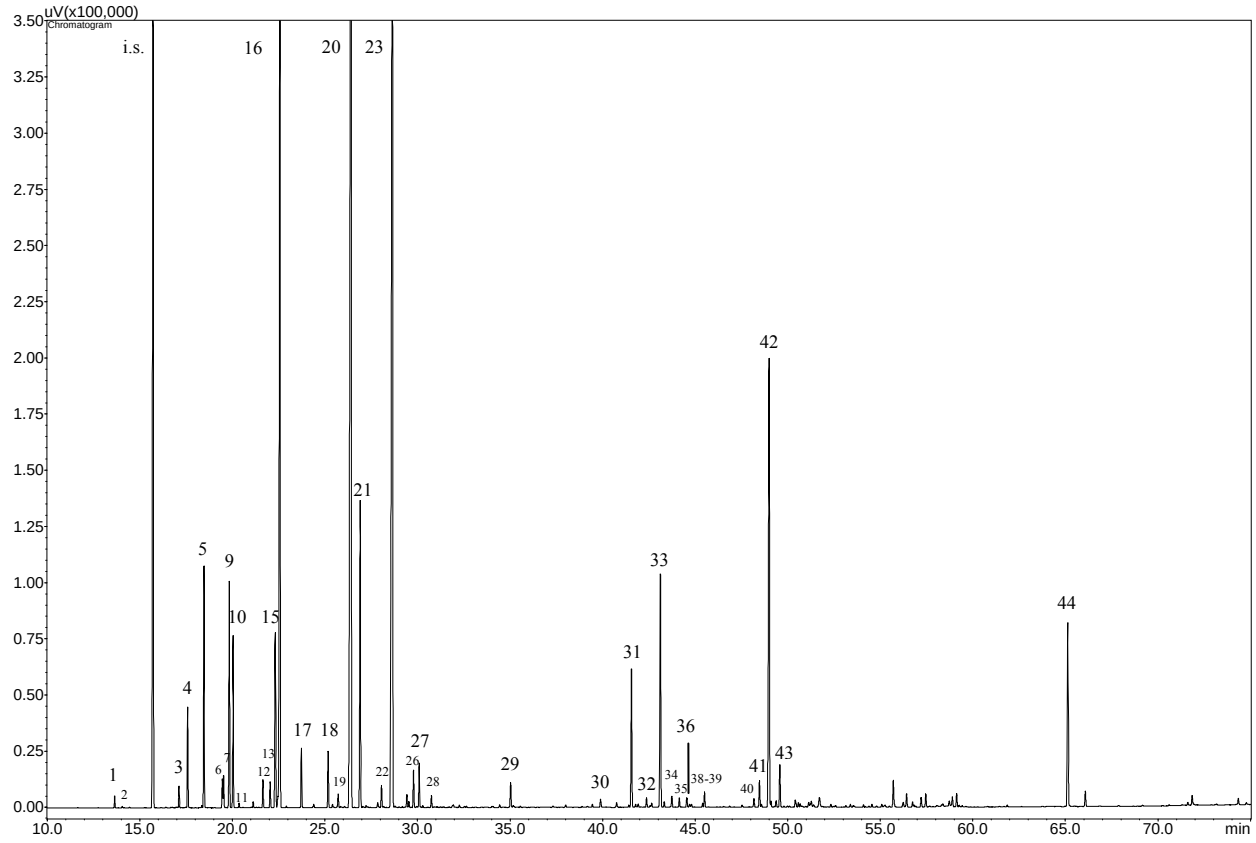

Figure 1: GC-FID chromatogram of sage oil on the 100 m SLB-5ms column. For peak identification see Table 1.

In 1963, Van den Dool and Kratz introduced the concept of the Programmed Temperature Retention Index (PTRI), based on a linear relationship between the elution temperature of *n*-alkanes and their carbon number [13].

The LRI value, by itself, is not an unambiguous system of identification because different compounds might have the same LRI using the same column and the same temperature program. However, it is not probable that they present also the same mass spectrum. The experimental LRIs, measured by the software against the *n*-alkanes, were automatically matched with those reported in the FFNSC mass spectra database, allowing a very easy and reliable identification.

Two filters have been used as library search parameters: the minimum similarity percentage (90%) and a LRI range. Under these conditions, compounds with a high spectra matching but with a LRI value falling out from the range selected are automatically excluded from the list. The LRI allowance windows and the tolerance range have been calculated in our laboratory by means of multiple injections of compounds for testing the repeatability in a period of several months.

With the commonly used 30 meters columns, an LRI range of +/- 5 allowed us to easily identify the analytes. Using the 100 meter columns, it was observed a greater variation of the LRI values and the tolerance range was set to +/-15. Nevertheless, the improved separation degree given by the 100 m columns avoided many of the coelutions which occur on the 30 m ones. Thus it was possible to obtain higher MS spectra similarity, easing the identification.

Figures 1 and 2 show the GC-FID chromatograms obtained for the sage essential oil using the SLB-5ms (Figure 1) and Supelcowax-10 (Figure 2) 100 m capillary columns. The 400000 theoretical plates generated by these columns provided a high degree of separation in a reasonable total run time (~70 min), very similar to the 30 meters column run time (~55 min), that generate approximately 120000 theoretical plates.

In Figure 3-5 are reported, as examples, a comparison of 3 enlargements of the chromatograms that illustrate the different degree of separation obtained on both 30 meters and 100 meters columns. Figure 3A shows that, while β-phellandrene is totally coeluted with 1,8-cineole on the SLB-5ms 30 m column, in the analyses carried out on the 100 meter columns the peak corresponding to β-phellandrene is partially resolved between limonene and 1,8-cineole and identified with a mass spectra similarity value of 91% [Figure 3B]. The chromatogram obtained on the 30 meter SLB-5ms column, shows only one peak with

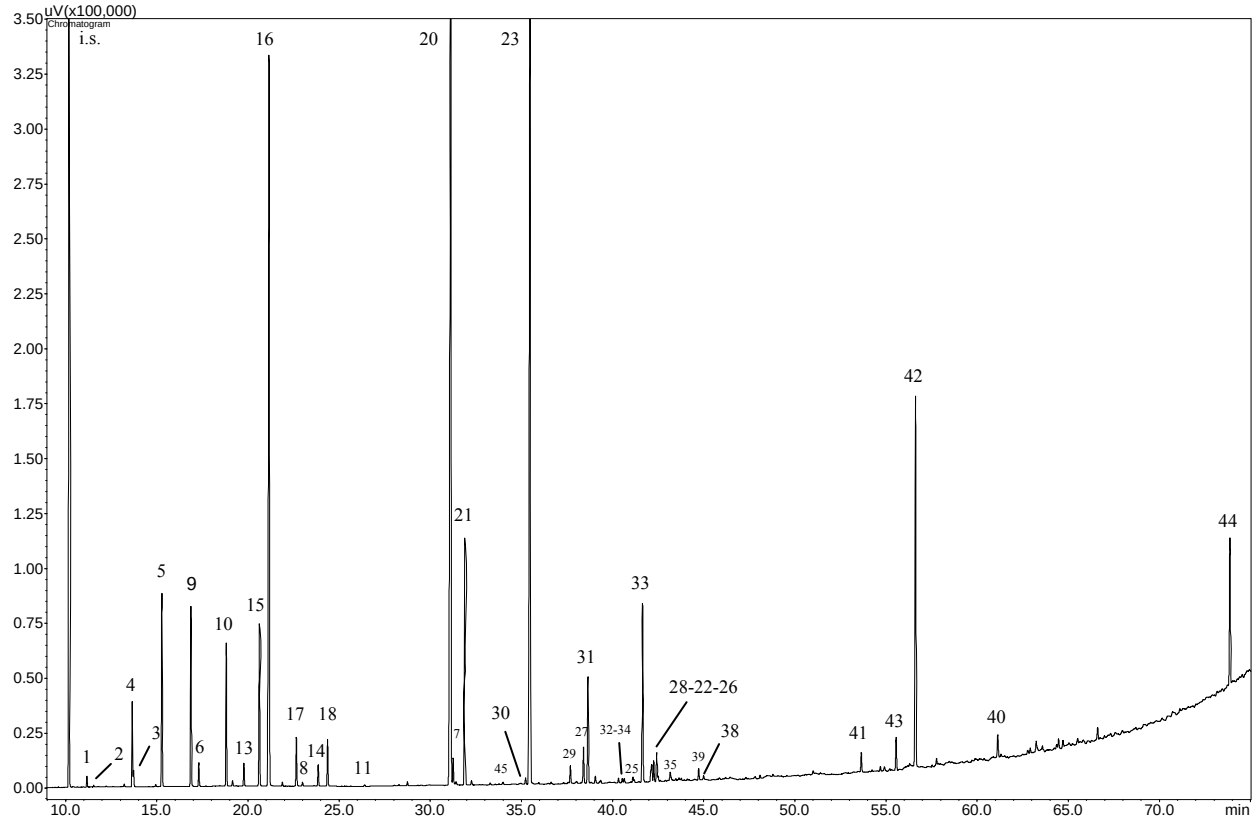

Figure 2: GC-FID chromatogram of sage oil on the 100 m Supelcowax-10 column. For peak identification see Table 1.

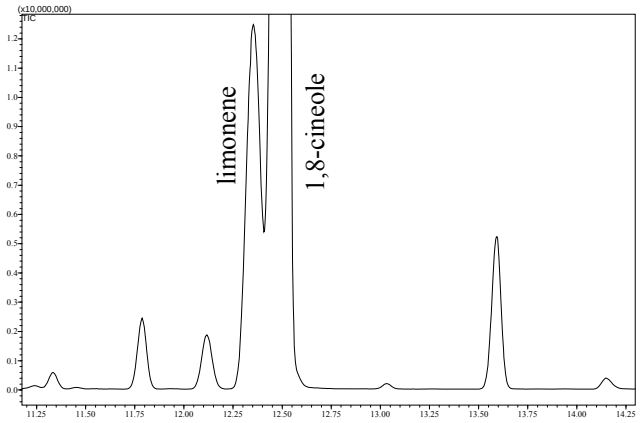

Figure 3A: Enlargement of the chromatogram on the SLB-5ms 30 m column related to peaks of limonene and 1,8-cineole.

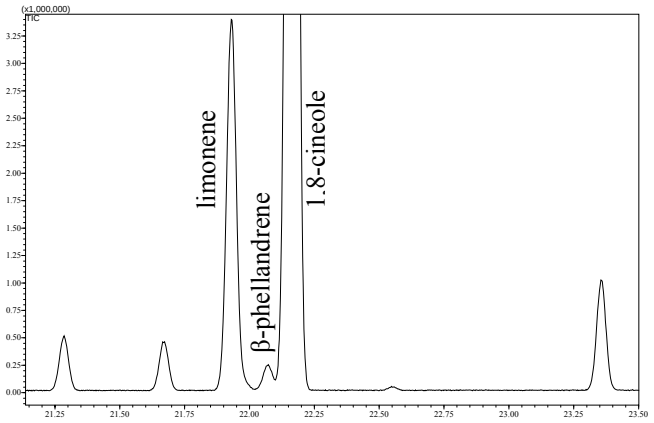

Figure 3B: Enlargement of the chromatogram on the SLB-5ms 100 m column related to peaks of limonene, β-phellandrene and 1,8-cineole.

LRI 978, identified as β-pinene [Figure 4A]. On the 100 m column, the chromatogram shows an additional peak, identified as 1-octen-3-ol with a mass spectra similarity percentage value of 96% [Figure 4B].Figures 5 illustrate an enlargement of a part of the chromatogram obtained on the Supelcowax-10 columns. The peak corresponding to α-phellandrene is clearly better separated in the chromatogram on the 100 m column (Figure 5B) then on the 30 m one (Figure 5A).

A total of 45 compounds were identified and listed according to their retention indices on the apolar column (Table 1). Major constituents of the oil, according to the data reported by Lawrence [14,15], resulted to be α-thujone, camphor, 1,8-cineole, viridiflorol, β-thujone, manool, α-humulene, camphene, limonene and β-pinene.

Quantification of analytes, was carried out by means of a GC-FID with the internal standard method. Due to the different response of the FID detector given by different

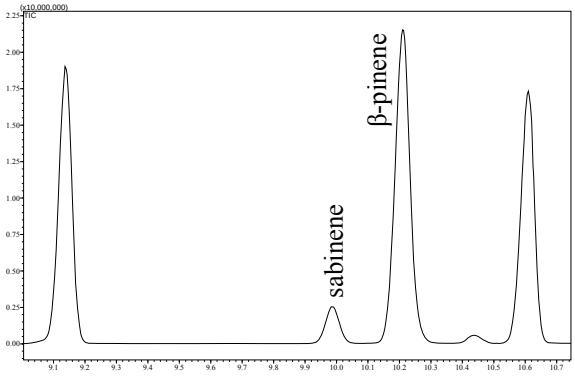

Figure 4A: Enlargement of the chromatogram on the SLB-5ms 30 m column related to peaks of sabinene and β-pinene.

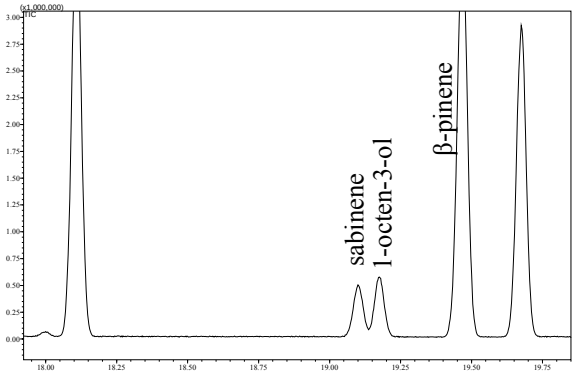

Figure 4B: Enlargement of the chromatogram on the SLB-5ms 100 m column related to peaks of sabinene, 1-octen-3-ol and β-pinene.

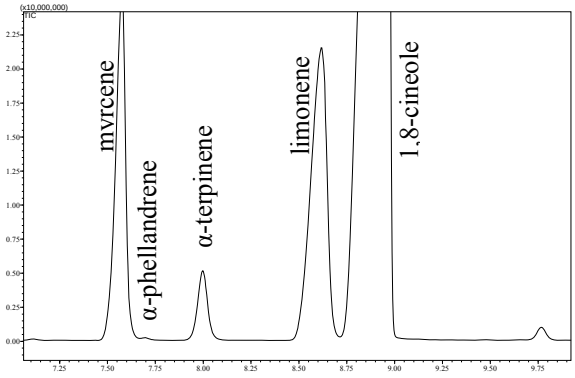

Figure 5A: Enlargement of the chromatogram on the Supelcowax-10 30 m column related to the peak of α-phellandrene.

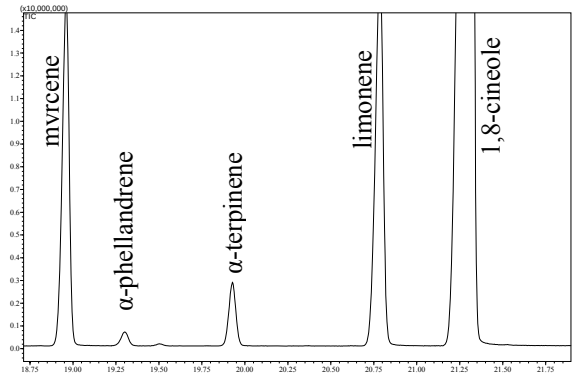

Figure 5B: Enlargement of the chromatogram on the Supelcowax-10 100 m column related to the peak of α-phellandrene.

Table 1: Qualitative and quantitative composition reported on both columns, listed in order of elution on the SLB-5MS.

	Compound	SLB-5ms			Supelcowax-10	
		LRI^{exp}	LRI^{lit}	Weight (g/100 g)	LRI^{exp}	Weight (g/100 g)
1	(*Z*)-Salvene	849	847	0.10	942	0.10
2	(*E*)-Salvene	859	856	0.02	953	0.02
3	α-Thujene	930	927	0.21	1033	0.17
4	α-Pinene	939	933	0.96	1031	0.95
5	Camphene	958	953	2.35	1079	2.27
6	Sabinene	978	972	0.28	1132	0.27
7	1-Octen-3-ol	980	978	0.42	1451	0.47
8	3-Octanone	986	-	-	1265	0.07
9	β-Pinene	986	978	2.28	1122	2.16
10	Myrcene	990	991	1.68	1169	1.66
11	3-Octanol	997	999	0.06	1394	0.06
12	α-Phellandrene	1012	1007	0.06	1177	0.06
13	α-Terpinene	1023	1018	0.28	1192	0.28
14	*p*-Cymene	1030	1025	0.28	1285	0.26
15	Limonene	1035	1030	2.11	1212	2.02
16	1,8-Cineole + β-Phellandrene	1040	1032	14.33	1224	14.03
17	γ-Terpinene	1064	1058	0.61	1258	0.62
18	Terpinolene	1092	1086	0.59	1297	0.57
19	Linalool	1102	1101	0.22	-	-
20	α-Thujone	1115	1100	27.14	1449	26.49
21	β-Thujone	1126	1118	4.35	1466	3.26
22	*trans*-Sabinol	1149	1140	0.35	1716	0.36
23	Camphor	1160	1149	21.84	1550	21.37
24	Thujol	1177	1169	0.20	1713	*
25	δ-Terpineol	1179	1170	0.09	1687	0.10
26	Borneol	1184	1173	0.53	1720	0.51
27	Terpinen-4-ol	1190	1180	0.63	1620	0.68
28	α-Terpineol	1204	1195	0.17	1711	*
29	Bornyl acetate	1293	1285	0.49	1602	0.44
30	β-Bourbonene	1399	1387	0.12	1544	0.09
31	β-Caryophyllene	1438	1424	1.61	1626	1.51
32	Aromadendrene	1457	1438	0.12	1636	0.11
33	α-Humulene	1473	1454	2.77	1700	2.54
34	Allo-aromadendrene	1479	1458	0.06	1675	0.07
35	γ-Muurolene	1489	1478	0.15	1739	0.19
36	Germacrene D	1498	1480	0.11	-	-
37	Viridiflorene	1508	1491	0.13	-	-
38	γ-Cadinene	1528	1514	0.05	1787	0.06
39	δ-Cadinene	1531	1518	0.18	1780	0.15
40	Spathulenol	1596	1476	0.12	2154	0.19
41	Caryophyllene oxide	1603	1587	0.48	2028	0.36
42	Viridiflorol	1616	1602	7.76	2118	7.01
43	Humulene epoxide II	1630	1613	0.78	2085	0.72
44	Manool	2057	2062	3.01	2062	2.81
45	α-Copaene	-	-	-	1514	0.05
24+28	α-Terpineol + thujol	-	-	-		0.50

Notes: LRI^{exp} are the experimental LRI obtained with the analyses; LRI^{lit} are the LRI reported in the FFNSC mass spectra library, used as references for the identification. Due to their larger variation, LRI^{lit} are not reported for the Supelcowax-10 column, as the results obtained on this column where used only as comparison. * Thujol and α-Terpineol are coeluted on the Supelcowax-10 column.

chemical classes of compounds, response factors have been applied to calculate absolute quantitative data. Response factors for each class of compounds have been previously calculated by means of multiple consecutive injections of standards, according to the method developed and reported in a previous work [16]. The amount of the analytes is expressed as g/100 g.

Quantitative values are reported in Table 1. Analyses have been carried out in triplicate, with CV% always lower than 5%. There is a good agreement between quantitative values obtained using the two different stationary phases.

Experimental

Extraction and sample preparation: Sage oil was obtained by hydrodistillation of fresh leaves using a Clevenger-type device for 4 hours. For qualitative purposes, the essential oil was diluted 1:100 (*v*/*v*) in hexane. For quantitative analyses, the sample (100 µL) was diluted in hexane to a final volume of 1 mL, after adding 100 µL of a nonane solution (10,000 ppm) as internal standard.

GC-MS: GC-MS analyses were carried out by means of a GCMS-QP2010 Plus (Shimadzu) system equipped with an AOC-20i autosampler. Applications were carried out using two different columns:

- SLB-5ms column (Supelco, Milan, Italy), 30 m x 0.25 mm I.D. x 0.25 µm film thickness and 100 m x 0.25 mm I.D. x 0.25 µm f.t.; stationary phase: silphenylene polymer, virtually equivalent in polarity to poly(5% diphenyl/95% methylsiloxane).
- Supelcowax-10 (Supelco), 30 m x 0.25 mm I.D. x 0.25 µm film thickness and 100 m x 0.25 mm I.D. x 0.25 µm f.t.; stationary phase: polyethylene glycol.

GC conditions were set as follows: injector split/splitless: 250°C; injection volume: 1 µL; head pressure: 26.7 kPa; carrier gas: He; linear velocity: 30 cm/sec (constant); split ratio: 1:200; oven temperature program: 50°C to 280°C at 3°C/min.

MS conditions were set as follows: ion source temperature was 220°C, interface temperature was 250°C, scan range was 40-400 *m/z*, with an acquisition frequency of 5 Hz. For mass spectral identification, the FFNSC 1.3 library (Shimadzu) [17] was basically used, along with Adams [18] and NIST08 [19] libraries. Identification was carried out by means of two filters: minimum similarity percentage (90%) and an LRI (linear retention indice) range (+/- 10 units). In order to determine LRI values, an *n*-alkane mixture (C_7-C_{30}) was analyzed under the same operational conditions of the sample. The experimental LRIs, measured by the software (calculated by means of the Van den Dool and Kratz equation), were automatically matched with those reported in the FFNSC mass spectra database.

GC-FID: GC-FID analyses were carried out by using a GC-2010 system (Shimadzu) equipped with the same columns as used for the GC-MS analyses.
Oven temperature program: 50°C to 250°C at 3°C/min, 250°C to 280°C at 10°C/min (5 min).
Injector and FID temperatures were set at 250°C and 280°C, respectively. Carrier gas was He, at a constant linear velocity of 30.0 cm/s and an initial head pressure of 99.8 KPa. FID conditions: sampling frequency: 12.5 Hz. Data were processed through the GCsolution (Shimadzu) software.

Quantitative analysis was carried out with the internal standard method, using response factors calculated for each chemical class, by means of the following equation: $\text{Weight\%}_a = ((A_a/A_{i.s.}) \times C_{i.s.} \times \text{RRF}) / \text{Weight}_{oil} \times 100$. Analyte amounts are expressed as g/100 g of oil. (A_a: peak area of the analyte; $A_{i.s.}$: peak area of the internal standard; $C_{i.s.}$: internal standard concentration (g/g); RRF: relative response factor).

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