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Application News

GC-MS

Reliable identification in GC-MS analysis with two independent analytical information

No. SCA_280_088

▪ Introduction

Main flavour and fragrance components prior to gas chromatographic (GC) separation are usually identified with mass spectrometry (MS) using spectral comparison and literature data. In the GC-MS peak identification of complex samples, the main difficulty is related to the almost identical mass spectra of terpenes. Hence, MS identification should be accompanied by additional information, namely retention index (RI), that can support the MS similarity search. There are several ways to calculate the RI of an unknown compound according to the specific chromatographic conditions [1-4]. The widely accepted way for programmed temperature retention index, denominated also as linear retention index (LRI), calculation is expressed as follows [4, 5]:

$$LRI = 100n\left(\frac{t_{Ri} - t_{Rz}}{t_{R(z+1)} - t_{Rz}}\right) + 100z$$

where t_R is the retention time, z is carbon atom number, i is the peak of interest, n is the difference in carbon atom number of the two standards (*n*-alkanes: n=1, even or odd fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs): n=2).
The Flavour and Fragrance Natural and Synthetic Compounds Library (FFNSC) is a comprehensive database containing mass spectrum of about 3500 individual flavour and fragrance molecules, acquired mainly from pure standards. Moreover, for each compound embedded experimental LRI values on three different stationary phases (SLB-5 ms, Equity-1 and Supelcowax-10) are also reported. Boosting the MS similarity search with simultaneous LRI filter of GCMSsolution a fast and reliable peak assignment can be achieved. Using two different types of stationary phases and the MS spectra and exploiting the multi-LRI feature of the FFNSC Library the identification will not be tentative, but confirmed by three individual analytical information.
The FFNSC Library can be an excellent support and applied in essential oil characterization, food aroma profile analysis, determination of fragrances in non-food matrix (e-liquids, cosmetics). In this application note some examples are reported.

▪ Analytical methods and identification

Flavour and fragrance extraction
The aroma and flavour constituents can be obtained in different ways according to the sample matrix. The easiest method is the dilute-and-shoot (D&S), what can be used in the case of essential oils and perfumes which contain mainly volatile compounds. Otherwise from solid or highly viscous matrices including also a huge amount of non volatile constituents solid-phase microextraction (SPME) can be suitable.

GC–MS analysis
The GC-MS profiles were acquired by a Shimadzu GCMS-QP2010ultra. For the separation an SLB-5 ms column and a Supelcowax-10 (30 m × 0.25 mm id × 0.25 µm film, Merck Millipore, Darmstadt, Germany) were employed.
GC parameters: The injection was performed at 280 °C in split mode in D&S method and at 270 °C in splitless mode in SPME analysis. In the latter case the fiber desorption time was 1 min. Constant linear velocity: 30 cm/sec (He). Temperature program: 40 °C (1 min, only in SPME analysis) to 280 °C (5 min), at 3 °C/min.
MS parameters: the samples were analysed in full scan mode using a mass range of 40-350 m/z; spectra generation frequency: 10 Hz; interface and ion source temperatures were 250 °C and 220 °C, respectively. MS ionization mode: electron ionization.
For LRI calculation on non-polar stationary phase a C_7-C_{30} saturated *n*-alkane (49451-U), while on polar stationary phase a C_4-C_{24} even FAMEs (49453-U) series were used as reference standard.

Volatile identification

Volatiles were identified using mainly the FFNSC Library ver. 3.0. The GCMSsolution can exploit the LRI features included in the FFNSC Library performing a reliable search process. The analyst can select the level of confidence to achieve by selecting different filter parameters. In fact, either a simple MS search can be performed or it can be supported by a second filter, namely LRI. To demonstrate the importance of the LRI approach in the first step only mass spectral similarity was used: even setting the MS filter more than 90%, the list of candidates obtained for the target molecule was quite long in many cases, and the candidate with the highest similarity match was not always the right one, as shown in Figure 1 (left upper). Using the adequate settings in both filter parameters, such as ≥ 90% MS similarity and ±5 LRI units, in every case a unique match was achieved, allowing a very fast and reliable peak assignment during the identification process (Figure 1, left down).

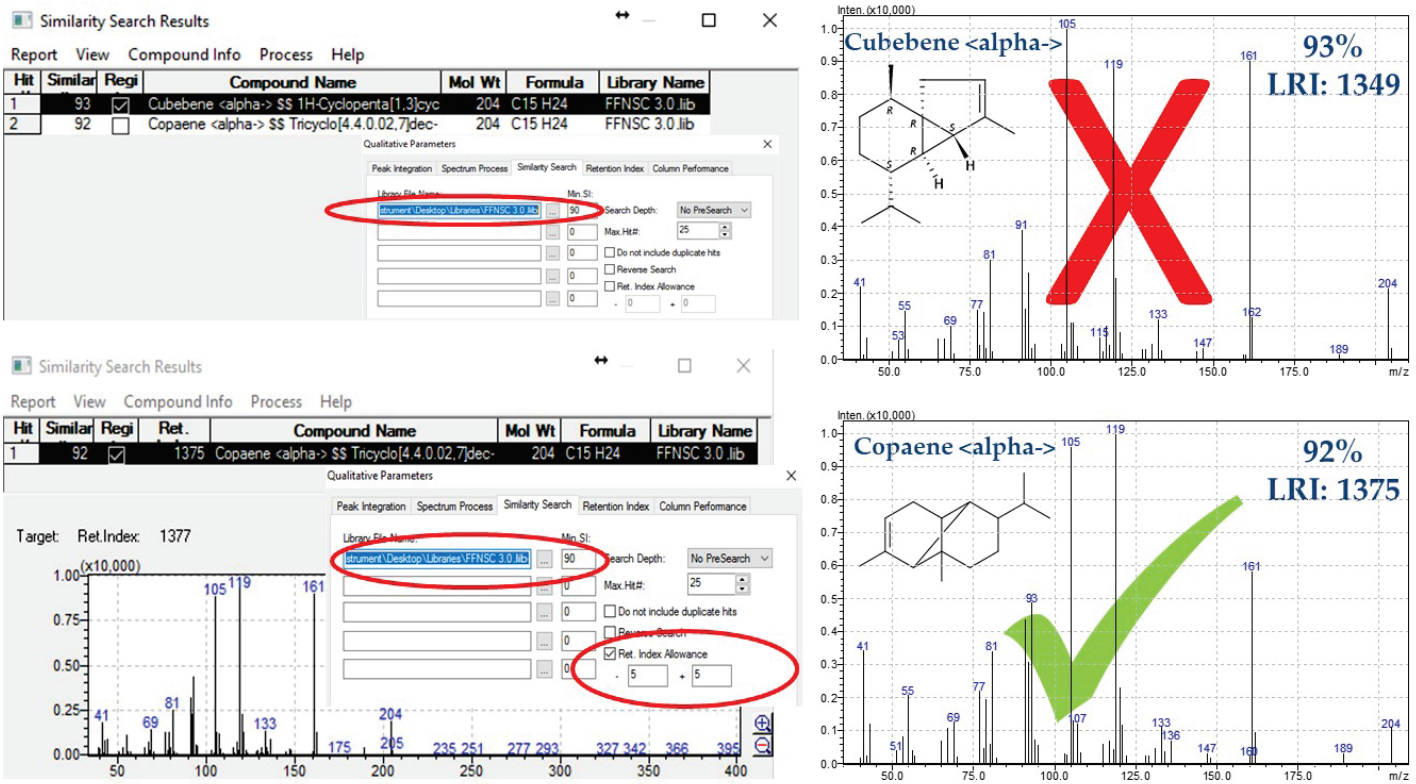

Figure 1: GCMSsolution automatic MS similarity (left, upper) and simultaneous LRI (left, down) filters

▪ Determination of geographical origin of pistachios

In this study the aroma and flavour constituents of eight pistachio (*Pistacia vera* (Figure 2) samples with different geographical origin were investigated by means of SPME–GC–MS analysis.

Sample preparation

Fresh pistachio kernels ((Iranian (green, normal), Turkey (green, red, mawardi), Greek, Italian, and Californian (primary and secondary quality)) were obtained from local certified cultivars and collected in the same period and maturation stage.

The isolation of the volatile fraction was carried out using a 1 cm 50/30 µm SPME fibre coated with divinylbenzene / carboxen / polydimethylsiloxane (DVB/CAR/PDMS) (Merck Millipore, Darmstadt, Germany). 2.5 g of each freshly and finely ground sample was placed in a 20 mL head-space vial and the vial was tightly capped immediately with a screw cap with PTFE/silicone septa. Each sample was extracted and analysed in triplicates. The samples were maintained at the extraction temperature, at 40 ºC, during thermal pre-equilibration for 30 and stirred at 160 rpm. The extraction of volatiles was carried out at 40 ºC for 60 min. Prior to use the fibre was conditioned in the GC injection port at 270 ºC in splitless mode for 1 h and controlled for interference using the same temperature program. Blanks of the vials were also performed.

Characterisation of pistachio volatiles

In this study eight pistachio samples were analysed. The volatile fraction profiles were obtained by means of GC-MS analysis as previously described. The identification of the flavour and fragrance compounds was carried out using mainly the FFNSC library.

Figure 2: *Pistacia vera* kernels in shells

Overall, in the eight pistachio samples a total of 167 aroma constituents were identified. Chromatogram of Californian pistachio sample is shown in Figure 3.

The aroma compounds belong to 13 different chemical classes, namely organic acids, linear and aromatic hydrocarbons, alcohols, aldehydes, esters, ethers, ketones, hydrocarbon and oxygenated monoterpenes, sesquiterpenes, pyrrole derivatives, and thio-compounds. In all pistachio samples the main class (58-87%) was the monoterepene class, in total 25 individual monoterpene type molecules were identified. Three of them, α-pinene, limonene and α-terpinolene, were characteristic in all pistachio samples, however their distribution differed significantly. In the Iranian and Californian pistachios the main constituent was limonene, while in pistachios from the Mediterranean zone (Turkey, Italy, Greece) it was less present (1-7%). In Sicilian and Greek kernels the most significant aroma constituent was α-pinene (51-57%), while α-terpinolene was present in low quantity. In the volatile profile of the Turkish pistachios, apart of the three characteristic molecules other monoterpenes were present, namely camphene, β-pinene, myrcene, δ-3-carene, α-terpinene, p-cymene, and p-cymenene, all over 3%.

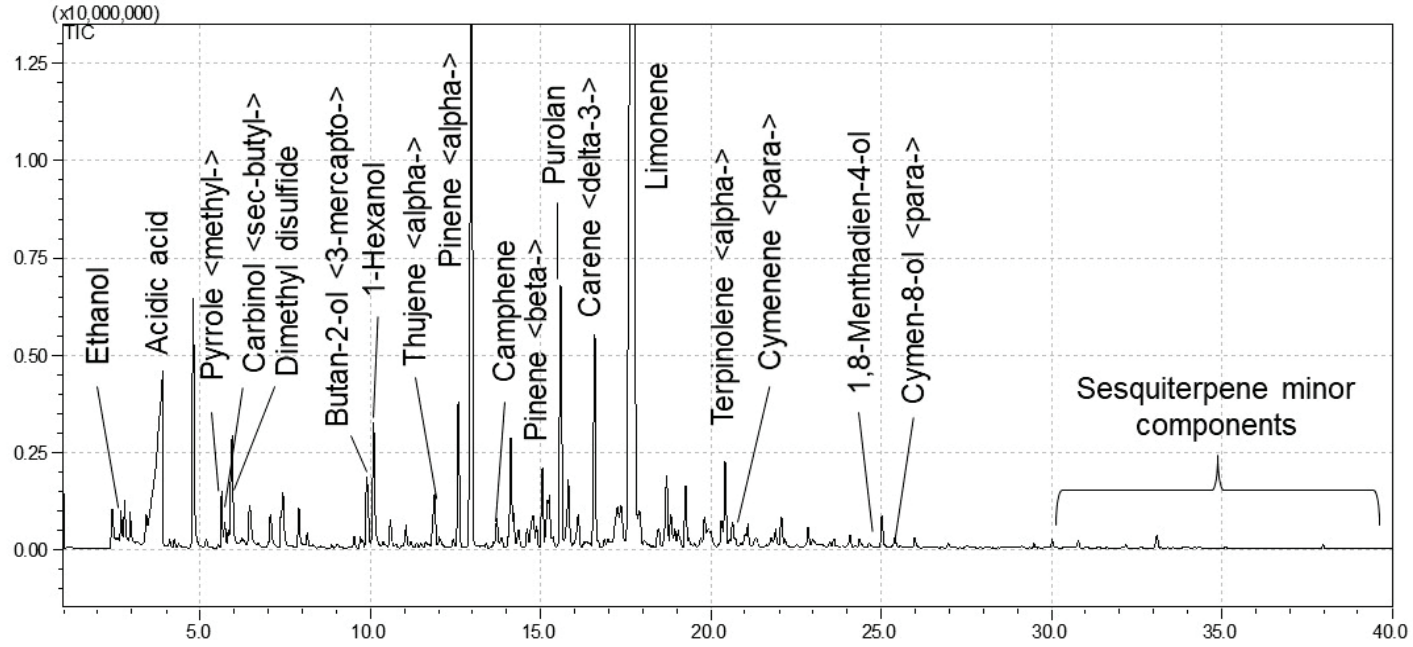

Figure 3: GC-MS chromatogram of Californian pistachio on SLB-5ms stationary phase

The Californian pistachio had the most complex profile, not only for the monoterpene fraction but also for the total volatile profile. Organic acids, alcohols, hydrocarbons, ketones, and pyrrole derivatives also play important role in the aroma composition. Among the acids, acetic acid was present in major quantity in all pistachio samples, especially in the Californian one (9%).

In total 26 alcohols were found in the samples from different origins, Turkish mawardi and the Sicilian samples presented the richest alcoholic profile. Greek, Turkish red and green samples were rich in hydrocarbons, mainly in octane and purolane. All pistachio samples had a notable presence of ketones, mainly 2-propanone and γ-butyrolactone.

Between the pyrrole derivatives methyl-pyrrole was present in high percentage (16-23 %) in Iranian normal and in Sicilian kernels. Monocyclic aromatic hydrocarbons and thio-compounds were characteristic in Californian pistachio; however, 2-thiopropane was present only in the two Iranian and Turkish mawardi samples. Aldehydes and the aromatic hydrocarbon naphthalene were typical in Turkish green pistachios.

The high number of identified compound provide the possibility of an unsupervised Principal Component Analysis (PCA). According to the obtained plots a good discrimination can be achieved in the geographical origin.

▪ Characterisation of *Cymbopogon martinii* essential oil

In this study the essential oil obtained from palmarosa (*Cymbopogon martinii*, Nepal, (Figure 4)) was studied by a dilute-and-shoot GC–MS method on non-polar and polar stationary phases (Figure 5 (a) and (b)).

Figure 4: *Cymbopogon martinii*

Sample preparation

Palmarosa essential oil was extracted with steam distillation. Hexane (purity > 97 %) was used to dilute the sample. The sample was injected in two different concentrations three times, to maximize the number of the identifiable compounds.

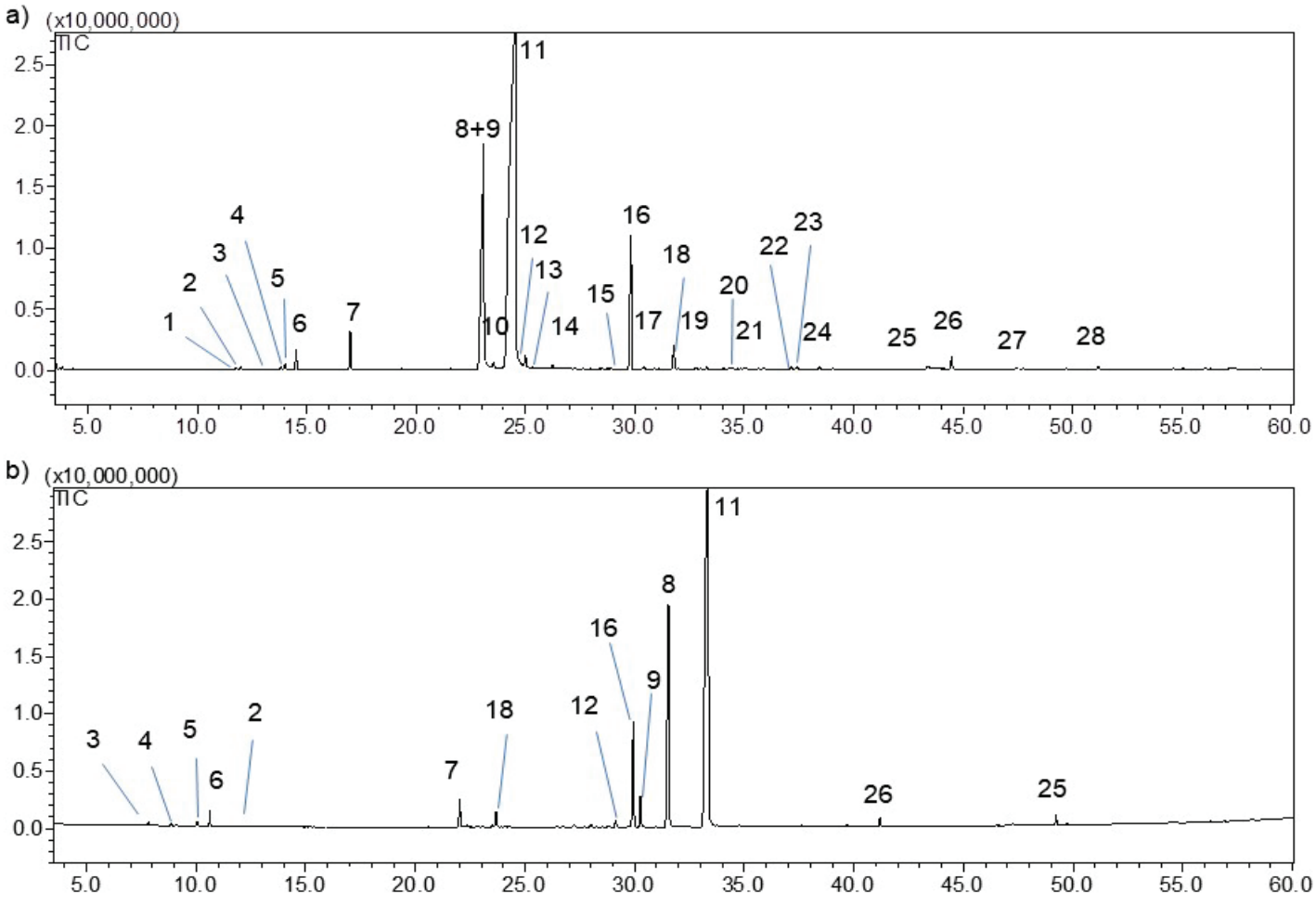

Figure 5: *GC-MS chromatogram of Cymbopogon martinii essential oil on SLB-5ms (a)* and on Supelcowax-10 *(b)*

Volatile profile

In the palmarosa oil 28 individual compounds were found and identified by the FFNSC MS spectral library. They are listed in Table 1, reporting the similarity match, the experimental and library LRI and their difference on both stationary phases. Thanks to the dual-column analysis the coelutions were resolved on one of the used stationary phases. Due to the „multiLRI" - MS similarity search 16 compounds were confirmed and other 12 tentatively identified.

Using the peak areas obtained by GCMS analysis relative area percentages were calculated, without any correction. The main components of the analysed palmarosa oil were geraniol (74.1%), nerol (~16%) and trans-geranyl acetate (3.94%), while β-ocimene was a minor compound (~1%) with an *E*/*Z* isomer ratio of ~80%. The obtained results are partially in accordance with the literature data due to the higher nerol content and the presence of geranyl octanoate [6, 7].

Table 1. Identified compounds of *Cymbopogon martinii* essential oil

N.	Compound name	MS similarity %	SLB-5 ms			Supelcowax-10			*Cymbopogon martinii* essential oil
			LRI_{FFNSC}	LRI_{exp}	ΔLRI	LRI_{FFNSC}	LRI_{exp}	ΔLRI	area%
1	Sabinene	98	972	973	-1	-	517	-	tr
2	Hept-5-en-2-one <6-methyl->	97	986	983	3	746	746	0	0.04
3	Myrcene	95	991	988	3	567	568	-1	0.10
4	Limonene	97	1030	1030	0	608	609	-1	0.07
5	Ocimene <(*Z*)-, beta->	96	1035	1036	-1	-	642	-	0.14
6	Ocimene <(*E*)-, beta->	97	1046	1046	0	-	658	-	0.53
7	Linalool	97	1001	1000	1	956	955	1	1.07
8	Nerol	92	1229	1230	-1	1189	1199	-10	16.93
9	Citronellol	96	1232	1231	1	1166	1167	-1	
10	Neral	97	1238	1239	-1	1086	1088	-2	0.17
11	Geraniol	95	1955	1955	0	1232	1240	-8	74.10
12	Geranial	96	1971	1968	3	1135	1138	-3	0.41
13	cis-Geranyl formate	94	1276	1278	-2	-	1075	-	0.02
14	trans-Geranyl formate	94	1300	1298	2	1110	1108	2	0.11
15	cis-Geranyl acetate	97	1361	1358	3	1125	1128	-3	0.03
16	trans-Geranyl acetate	96	1380	1379	1	1159	1157	2	3.94
17	Elemene <beta->	92	1390	1392	-2	-	992	-	0.07
18	Caryophyllene <(*E*)->	96	1424	1424	0	-	996	-	0.66
19	Humulene <alpha->	97	1454	1458	-4	-	1067	-	0.05
20	Germacrene D	96	1480	1484	-4	-	1093	-	0.04
21	Cadinene <delta->	94	1518	1520	-2	-	1152	-	0.03
22	Geranyl butyrate	97	1559	1555	4	1290	1291	-1	0.06
23	Nerolidol <(*E*)->	96	1561	1562	-1	1431	1432	-1	0.04
24	Caryophyllene oxide	94	1587	1588	-1	1364	1371	-7	0.03
25	Farnesol <(2*E*,6*E*)->	96	1716	1718	-2	-	1735	-	0.11
26	Geraniol hexanoate	97	1748	1749	-1	-	1478	-	0.24
27	Farnesyl acetate	96	1832	1833	-1	-	1644	-	0.03
28	Geranyl octanoate	96	1943	1944	-1	1667	1669	-2	0.05
	Total								**99.08**

* coeluted with previous peak

▪ Fragrances in real cosmetic sample

Volatile profile of a real shampoo sample (Figure 6) was studied by SPME–GC–MS method.

Sample preparation

A representative volatile profile was obtained using a 1 cm 50/30 µm DVB/CAR/PDMS SPME fibre. 1 g of shampoo sample was placed in a 10 mL head-space (SPME) vial and the vial was tightly

Table 2. Identified compounds of real shampoo sample

	Compound name	LRI_{FFNSC}	LRI_{exp}	ΔLRI	MS similarity %	Area %
1	Acetone	628	619	9	96	0.79
2	Pinene <alpha->	933	933	0	94	0.03
3	Heptanone<5-methyl-3->	938	938	0	97	1.52
4	Pinene <beta->	978	979	-1	96	0.12
5	Hept-5-en-2-one <6-methyl->	986	984	2	98	0.06
6	Myrcene	991	989	2	99	0.40
7	Octanal <n->	1006	1004	2	98	0.08
8	Hexyl acetate	1012	1011	1	96	0.11
9	Cymene <para->	1025	1025	0	99	0.18
10	Limonene	1030	1030	0	99	6.60
11	Eucalyptol	1032	1033	-1	93	1.30
12	Ocimene <(E)-, beta->	1046	1046	0	97	0.18
13	Terpinene <gamma->	1058	1059	-1	96	0.11
14	Dihydromyrcenol	1081	1077	4	98	28.25
15	Linalool oxide <trans->	1088	1088	0	91	0.19
16	Linalool	1101	1103	-2	98	19.96
17	Nonanal <n->	1107	1106	1	96	0.12
18	Rose oxide	1112	1112	0	91	0.07
19	Phenethyl alcohol	1113	1114	-1	96	0.04
20	Octanoate <methyl->	1125	1123	2	97	1.13
21	Camphor	1149	1149	0	99	1.98
22	Menthone	1158	1157	1	98	0.18
23	Benzyl acetate	1167	1163	4	96	0.05
24	Isoborneol	1165	1165	0	93	0.39
25	Borneol	1173	1174	-1	98	0.35
26	Terpinen-4-ol	1184	1182	2	92	0.06
27	Dodec-1-ene	1191	1192	-1	96	0.19
28	Terpineol <alpha->	1195	1197	-2	96	1.56
29	Estragole	1201	1199	2	94	0.22
30	Dodecane <n->	1200	1200	0	99	1.61
31	Dodecene, <(2Z)-2->	1205	1205	0	99	0.78
32	Decenal <n->	1208	1207	1	98	0.08
33	Neral nitrile	1220	1220	0	93	0.05
34	Nerol	1229	1226	3	93	0.55
35	Oct-7-enol <3,7-dimethyl->	1228	1228	0	99	1.82
36	Acetic acid <(3-methylbutoxy)-, 2-propenyl-> ester	1243	1238	5	93	4.95
37	Carvone	1246	1246	0	91	0.07
38	Linalyl acetate	1250	1250	0	99	2.65
39	Geraniol	1255	1251	4	94	0.56
40	Dihydrolinallyl acetate <beta-, gamma-, (±)->	1275	1271	4	92	0.32
41	Decyl alcohol	1278	1274	4	97	0.08
42	Isobornyl acetate	1287	1289	-2	98	6.84
43	Undecan-2-one	1294	1293	1	91	0.01
44	Tridecane <n->	1300	1300	0	98	0.09
45	Undecanal <n->	1309	1308	1	99	0.11
46	Decanoate <methyl->	1327	1323	4	97	0.49
47	Dihydrocarvyl acetate	1325	1330	-5	91	0.05
48	Terpinyl acetate <alpha->	1349	1348	1	99	1.80
49	cis-Geranyl acetate	1361	1358	3	97	0.49
50	Cyclohexanol <4-tertbutyl-> acetate	1368	1368	0	98	0.39
51	trans-Geranyl acetate	1380	1378	2	98	0.99
52	Tetradec-1-ene	1392	1392	0	94	0.02
53	Tetradecane <n->	1400	1400	0	97	0.15
54	Dodecanal <n->	1410	1409	1	92	0.13
55	Bergamotene <alpha-, cis->	1416	1420	-4	93	0.58
56	Nopyl acetate	1423	1423	0	98	1.96
57	Cedrene <beta->	1423	1427	-4	92	0.25
58	Citronellylacetone	1435	1435	0	91	0.02
59	Thujopsene <cis->	1433	1437	-4	98	2.30
60	Himachalene <alpha->	1449	1454	-5	91	0.04
61	Acoradiene <beta->	1471	1467	4	92	0.02
62	Thujopsadiene <cis->	1467	1470	-3	96	0.05
63	Dodecanol	1476	1475	1	97	0.09
64	Pentadecane <n->	1500	1500	0	97	0.05
65	Adoxal	1505	1505	0	97	0.13
66	Cuparene	1513	1511	2	97	0.30
67	Dodecanoate <methyl->	1527	1524	3	96	0.73
68	Benzoic acid <2-[[[4-(4-hydroxy-4-methylpentyl)-, 3-cyclohexen-1-yl]methylene]amino]-, methyl-> ester	1589	1587	2	98	0.22
69	Cedrol	1610	1613	-3	91	0.04
70	Dihydrojasmonate <methyl-, cis->	1650	1650	0	96	0.09
71	Vertofix coeur	1765	1770	-5	98	0.19
72	Galaxolide	1845	1845	0	97	0.20
73	Tetralide	1855	1854	1	92	0.08
	Total					98.64

capped immediately with a screw cap with PTFE/silicone septa. Each sample was extracted and analysed in triplicates. The samples were maintained at the extraction temperature, 30 ºC, during thermal pre-equilibration for 30 min at 200 rpm. The extraction of volatiles was carried out at 30 ºC for 15 min.

Volatile profile

In the analysed shampoo sample 73 synthetic and natural fragrance compounds were identified using two individual analytical information, namely LRI and MS similarity. The fragrances are listed in Table 2. In the most cases very high spectral similarity was obtained, but in the case of peaks with lower intensity and poorer spectral quality the confirmation of LRI values is even more important.

Using simultaneous LRI – MS filters a unique candidate was obtained for each target compound, covering more than 98% of the total volatile fraction detected on the chromatogram (Figure 7).

Figure 6: Hair care products

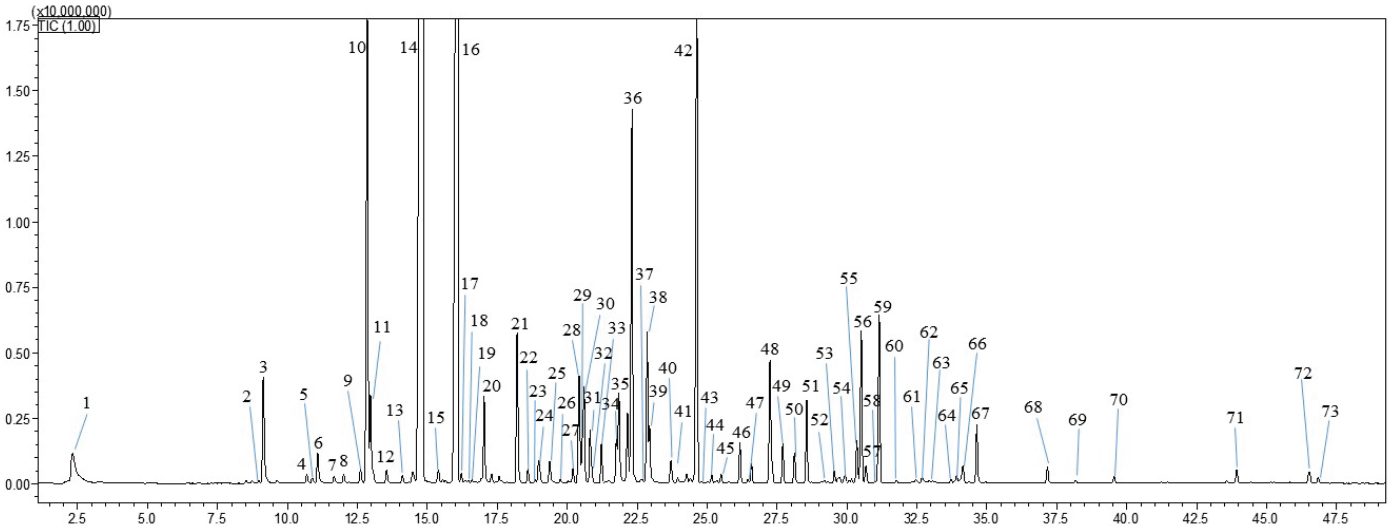

Figure 7: SPME-GC-MS chromatogram of shampoo on SLB-5ms stationary phase

▪ Conclusion

Analysis of foodstuff, essential oil and hair care product by means of GC-MS was carried out. The support of the FFNSC Library, with embedded LRI, provided a rapid and reliable determination of the main components. Using the MS similarity over 90% and a ±5 LRI range filters simultaneously, a unique match was achieved for the compounds. The proposed approach can be effectively used for authentication and characterisation.

▪ References

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