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Application Note

GC-FID Method with Nitrogen as Carrier Gas for Simple-Routine Analysis of Essential Oils

Plant Product Authentication to Adulteration Testing

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Abstract

The aim of this research is to explore the performance of nitrogen as an alternative carrier gas in gas chromatography-flame ionization detection (GC-FID) for routine analysis of the essential oils. For this purpose, a bergamot (*Citrus bergamia)* essential oil was used. Helium is the most frequently used, but its shortage or slow supply has led to investigations of hydrogen (H_2) and nitrogen (N_2) as alternative carrier gases. But if on the one hand important precautions must be taken when H_2 is selected as carrier gas, from other N_2 has the lowest optimal linear velocity, thus slow analysis times are registered. However, a slightly higher linear velocity (20 cm/s) than the optimal one (about 10 cm/s) was employed allowing to obtain comparable helium-based GC-FID analysis time. The developed method allowed the quantification of 67 terpene compounds including monoterpenes, sesquiterpenes, and oxygenated derivatives in bergamot essential oil. All components were eluted in about 47 min. In such a respect, an automatic peak attribution model based on the use of retention times and linear retention index (LRI) values was optimized avoiding mistaken peak attributions

1. Introduction

Gas chromatography-flame ionization detection (GC-FID) is one of the most popular analytical techniques widely used in many different areas, including flavor and fragrance industries.[1] Reasons for the popularity of GC-FID include large variety of analyzable compounds, uniform and sensitive response to volatile and semivolatile carbon-containing compounds, wide applicability coupled to fast response, large dynamic range, and low cost.[9]

Almost all GC-FID applications employ helium as carrier gas due to its chemical properties such as inertia that yields optimal chromatography while minimizing undesirable reactions.[5] However, helium shortage or its slow supply has led to investigations of hydrogen (H_2) and nitrogen (N_2) as alternative carrier gases for GC analyses. The use of H_2 provides analysts with benefits like increasing of the analysis speed, thus increasing the throughput of the laboratory, and cost saving considering that the price of H_2 is significantly lower than that of helium. On the other hand, a set of problems is related to the use of H_2 in GC; it results particularly reactive and can degrade certain compounds, especially the most susceptible.[6] Its high reactivity mainly consists in the capability to reduce nitro groups in explosive and other nitro compounds, to active silanol groups present on the surface of GC inlet liners, thus becoming reactive, and to hydrogenate the double bonds of components at high GC injector temperatures.[6,7] Also, specific precautions are necessary when H_2 is used in lab. An accurate quantification is compromised in GC-FID if the aforementioned events occur because the native chemical composition of target compounds is altered.

The best alternative to helium as a GC-FID carrier gas seems be N_2 due to its inertness, readily available (can be generated in-situ using a generator), low cost, and safety. However, N_2 is known to have the lowest optimal linear velocity (about 10 cm/s), thus slow analysis times are registered.[2] In addition, N_2 has a much steeper Golay curve than helium and H_2 gases, thus the separation efficiency decreases significantly as the flow rate increases. Nevertheless, there are key elements that indicate N_2 as a suitable and effective

carrier gas for conventional and simple-routine GC-FID analyses. For example, it should be noted that a slightly higher linear velocity (e.g., 20 cm/s) than the optimal one can reduce analysis time, although the efficiency is reduced. For this reason, it becomes fundamental to select the most appropriate stationary phase offering very high selectivity to counterbalance the efficiency loss. The aim of this research is to explore the performance of N_2 as an alternative carrier gas in essential oil analyses by using GC-FID instrumentation.

For this purpose, a bergamot (*Citrus bergamia)* essential oil was used. Particular attention was also paid in the development of an automatic peak attribution model based on the use of retention times (RT) and linear retention index (LRI) for the exact quantification of volatile components grouped in monoterpene, sesquiterpene, and oxygenated derivatives including aldehydes, ketones, alcohols, esters.

2. Experimental

2.1 Samples, chemicals, and sample preparation

A C_7-C_{30} saturated alkanes (1000 µg/mL) standard mixture in n-hexane was utilized for determining LRIs and RTs. A bergamot (*Citrus bergamia)* essential oil was kindly supplied by S. Gatto S.r.l. (Messina, Italy) for the construction of an FID-database containing reference compounds, while a bergamot essential oil was extracted in the laboratory through manual pressure applied to the fruit (collected in Calabria) peels. Before analysis, both essential oils (50 µL) were solubilized in 950 µL of n-heptane (dil. 1:20) and injected in the GC-FID instrument.

2.2 GC-FID analysis of the cold-pressed peels bergamot essential oil

The separation and detection of monoterpenes, sesquiterpenes, and oxygenated derivatives in cold-pressed peels bergamot essential oil was performed by using GC-FID (**Table1**).

Table 1. GC-FID conditions used for the analysis of cold-pressed peels bergamot essential oil

GC-FID Parameters	
Instrument:	Nexis GC-2030 high-performance capillary gas chromatograph (Shimadzu Europe, Germany) equipped with an FID detector. A split-splitless injector and an automatic AOC-20i autosampler were installed on the GC instrument.
Column:	SLB®-5ms 30 m × 0.25 mm, 0.25 µm (**28471-U**)
Oven:	50 °C to 250 °C at 3 °C/min
Injection temp.:	300 °C
Initial inlet pressure:	59.1 kPa
Carrier gas:	N_2 at 20 cm/s of linear velocity (constant)
Injection volume:	0.5 µL with a split ratio of 1:10
Detector:	FID 320 °C; Sampling rate: 40 ms; Gasflows: 40 mL/min for H_2, 10 mL/min for the make-up gas (N_2), and 400 mL/min for air

A reference homolog series of C_7-C_{30} saturated alkanes was used for RTs determination. Data were collected and processed using the LabSolution software (version 5.93, Shimadzu). The peak assignment was carried out in automatic manner using a lab-constructed FID database based on the use of the Automatic Adjustment Retention Time (AART) algorithm (LabSolution software, Shimadzu). Such a strategy allowed to determine the RTs of target compounds from LRIs listed in the FID-database.

2.3 Lab-constructed FID database

The construction of the FID-database was made by the injection of the reference bergamot essential oil. The terpenes composition of the bergamot essential oil was established on the base of articles published since 1979.[3] The injection of the essential oil was carried out by using the same instrumentations and analytical conditions as before described. A C_7-C_{30} homolog series was used for the determination of LRIs. All reference components of the bergamot essential oil were listed in the FID-database, and the following data were included in the compound table: compound name, retention time along with the LRI value. All RT and LRI values were obtained at the maximum point of the chromatographic peak. An extract of the lab-constructed FID-database is illustrated in **Figure 1**.

ID#	Name	Ret. Time	Ret. Index
1	alpha-Thujene	9,630	927
2	alpha-Pinene	9,959	935
3	Camphene	10,597	951
4	Sabinene	11,480	975
5	beta-Pinene	11,755	981
6	6-methyl-Hept-5-en-2-one	11,930	986
7	Myrcene	11,930	992

Figure 1. An extract of the lab-constructed FID-database containing reference terpenes along with RT and LRI values.

3. Results and Discussion

3.1 GC-FID analysis of the cold-pressed peels bergamot essential oil

GC-FID chromatogram of the cold-pressed peels bergamot essential oil is shown in **Figure 2**. A total of 67 components grouped in different classes of terpenes, including monoterpene, sesquiterpene, and oxygenated derivatives (**Table 2**) were satisfactory resolved. All components were eluted in about 47 min, in accordance with analysis times (ca. 45 min) of bergamot essential oils obtained using helium as carrier gas in GC-FID analyses.[4] Although a higher linear velocity of the N_2 than optimal one was used, which means efficiency loss, the stationary phase used provided very high selectivity for the separation of terpene compounds. This means that the use of non-optimal linear velocities of carrier gas can determine the reduction of the resolving power, but a comprise between GC-FID run time and peak resolution should be considered.[9]

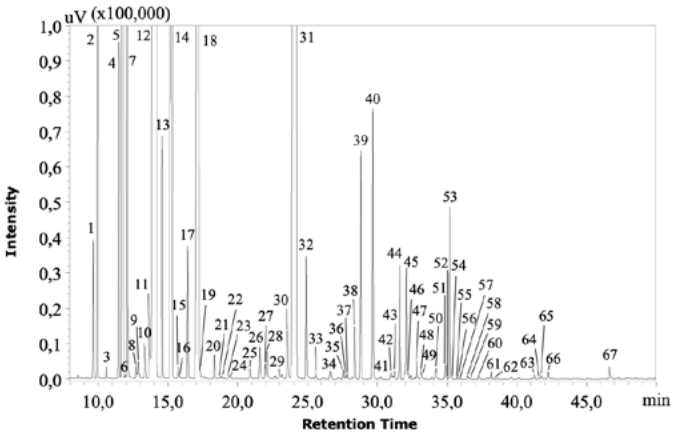

Figure 2. GC-FID chromatogram of the cold-presses peels bergamot essential oil obtained using N2 as carrier gas. See **Table 2** for peak assignment.

The FID is the detector of choice in GC analysis for quantitative purposes. It is a destructive mass-sensitive detector, and its response is proportional to the mass of carbon atoms that pass through it in unit time.[8] The FID gives unit response for most hydrocarbons; this allows to quantify components in mixtures without having calibration standards for every component. Amounts of components in a sample will be proportional to their peak areas. So, a simple area percent (%) report will closely reflect the mass percent of each component in a mixture. In **Table 2** are reported the concentrations expressed in % values of the terpene compounds quantified in cold-pressed peels bergamot essential oil. Although FID detector does not provide structural information of the analyzed molecules, relative retention data can be used as the primary criterion for peak assignment. In this research, we developed a lab-constructed FID-database to determine the RTs of target compounds by the injection of a reference bergamot essential oil. In the compounds table, all components were listed along with RTs and LRIs, the latter calculates by injecting the C_7-C_{30} homolog series. Using the AART algorithm, the LabSolution software was able to determine the RTs of the analytes in cold-pressed peels bergamot oil, starting from the RT and LRI values of the new C_7-C_{30} homolog series. This means that the retention times in FID-database were modified according to retention of the homolog series. Such strategy was able to quantify correctly all the terpene components avoiding mistaken peak attributions in automatic manner. No errors in peak assignment and quantification were highlighted indicating that such a strategy can be used as model for simple-routine GC-FID analyses of essential oils.

Table 2. Identity of the terpene compounds in cold-pressed peels bergamot essential oil. Abbreviation: RT: retention time (min); LRI: linear retention index. The terpenes contents are expressed in % values, tr indicates trace level.

ID	Name	Class	RT (min)	LRI	Content (%)
1	α-Thujene	Monoterpene	9.640	927	0.29
2	α-Pinene	Monoterpene	9.969	935	1.07
3	Camphene	Monoterpene	10.607	951	0.03
4	Sabinene	Monoterpene	11.490	975	0.88
5	β-Pinene	Monoterpene	11.775	981	5.03
6	6-methyl-Hept-5-en-2-one	Ketone	11.940	986	0.01
7	Myrcene	Monoterpene	11.950	992	1.85
8	n-Octanal	Aldehyde	12.706	1010	0.05
9	α-Phellandrene	Monoterpene	12.872	1012	0.05
10	α-Terpinene	Monoterpene	13.327	1020	0.14
11	p-Cymene	Monoterpene	13.729	1029	0.08
12	Limonene	Monoterpene	14.188	1036	48.10
13	(E)-, β-Ocimene	Monoterpene	14.588	1049	0.51

Table 2. (cont.) Identity of the terpene compounds in cold-pressed peels bergamot essential oil. Abbreviation: RT: retention time (min); LRI: linear retention index. The terpenes contents are expressed in % values, tr indicates trace level.

ID	Name	Class	RT (min)	LRI	Content (%)
14	γ-Terpinene	Monoterpene	15.278	1062	6.91
15	(Z)-Sabinene hydrate	Alcohol	15.726	1078	0.03
16	n-Octanol	Alcohol	15.826	1078	0.01
17	Terpinolene	Monoterpene	16.418	1090	0.30
18	Linalool	Alcohol	17.207	1107	8.38
19	n-Nonanal	Aldehyde	17.301	1112	0.03
20	neo-allo-Ocimene	Monoterpene	18.328	1132	0.06
21	(Z)-Limonene oxide	Alcohol	18.644	1136	0.00
22	(E)-Limonene oxide	Alcohol	18.835	1139	0.01
23	Camphor	Alcohol	19.362	1157	tr
24	Citronellal	Aldehyde	19.457	1159	0.01
25	Terpinen-4-ol	Alcohol	20.888	1188	0.04
26	α-Terpineol	Alcohol	21.587	1204	0.10
27	n-Decanal	Aldehyde	21.964	1212	0.04
28	octyl-Acetate	Ester	22.095	1216	0.08
29	Nerol	Alcohol	22.973	1241	0.04
30	Neral	Aldehyde	23.553	1248	0.19
31	Linalyl acetate	Ester	24.211	1259	22.44
32	Geranial	Aldehyde	24.907	1277	0.30
33	Bornyl acetate	Ester	25.614	1292	0.02
34	Nonyl acetate	Ester	26.648	1317	0.03
35	Geranate-methyl	Ester	27.570	1330	0.03
36	Linalyl propionate	Ester	27.721	1336	0.01
37	δ-Elemene	Sesquiterpene	27.844	1343	0.01
38	α-Terpinyl acetate	Ester	28.371	1353	0.14
39	Neryl acetate	Ester	28.817	1365	0.57
40	Geranyl acetate	Ester	29.677	1384	0.70
41	β-Elemene	Sesquiterpene	30.251	1400	0.01
42	Decyl acetate	Ester	30.981	1415	0.02
43	α-, (Z)-Bergamotene	Sesquiterpene	31.240	1417	0.02
44	(E)-Caryophyllene	Sesquiterpene	31.613	1424	0.31
45	α-, (E)-Bergamotene	Sesquiterpene	32.083	1437	0.30
46	Aromadendrene	Sesquiterpene	32.222	1439	0.02
47	(E)-, β-Farnesene	Sesquiterpene	32.822	1456	0.05
48	α-Humulene	Sesquiterpene	33.123	1460	0.03
49	β-Santalene	Sesquiterpene	33.199	1464	0.01
50	Germacrene D	Sesquiterpene	34.198	1486	0.04
51	(Z)-, α-Bisabolene	Sesquiterpene	34.866	1505	0.04
52	(E,E)-, α-Farnesene	Sesquiterpene	34.970	1508	tr
53	β-Bisabolene	Sesquiterpene	35.189	1511	0.45
54	(Z)-, γ-Bisabolene	Sesquiterpene	35.334	1515	0.01
55	δ-Cadinene	Sesquiterpene	35.684	1523	tr
56	β-Sesquiphellandrene	Sesquiterpene	35.839	1527	tr
57	(E)-, γ-Bisabolene	Sesquiterpene	35.969	1531	tr
58	(E)-, α-Bisabolene	Sesquiterpene	36.487	1545	0.01
59	(Z)-Sesquisabinene hydrate	Alcohol	36.652	1550	0.00
60	(E)-Nerolidol	Alcohol	37.277	1567	0.02

Table 2. (cont.) Identity of the terpene compounds in cold-pressed peels bergamot essential oil. Abbreviation: RT: retention time (min); LRI: linear retention index. The terpenes contents are expressed in % values, tr indicates trace level.

ID	Name	Class	RT (min)	LRI	Content (%)
61	Spatulenol	Alcohol	37.869	1593	0.01
62	Caryophyllene oxide	Alcohol	38.126	1600	0.01
63	Norbornarol	Alcohol	41.258	1668	0.01
64	epi-β-Bisabolol	Alcohol	41.578	1677	0.01
65	Campherenol	Alcohol	41.758	1682	0.01
66	α-Bisabolol	Alcohol	42.248	1695	0.02
67	Nootkatone	Ketone	46.628	1819	0.05
	TOTAL				**100.00**

4. Conclusion

The present research explored the performance of N_2 as alternative carrier gas in simple-routine GC-FID analyses of the essential oils. A cold-pressed peels bergamot essential oil was analyzed using N_2 at a constant linear velocity of 20 cm/s. Although the linear velocity was not optimal (about 10 cm/s), that means efficiency loss, the stationary phase used provided very high selectivity for the separation of 67 terpenes. All the components were eluted in about 47 min allowing to obtain comparable helium-based GC-FID analysis time. For an accurate peak attribution, an FID-database containing target terpenes, RT and LRI values was developed. Such strategy was able to attribute and to quantify correctly all the terpene components in automatic manner. No errors in peak assignment were highlighted indicating that such an approach can be used as model for simple-routine GC-FID analyses of essential oils.

Summary:

- Nitrogen (N_2) showed promise as an alternative carrier gas in gas chromatography-flame ionization detection (GC-FID) analysis of essential oils.
- The method using N_2 as the carrier gas achieved comparable analysis times to helium-based GC-FID, allowing for efficient analysis of essential oils.
- A total of 67 terpene compounds in bergamot essential oil were successfully quantified using the developed method.
- The study optimized an automatic peak attribution model based on retention times (RT) and linear retention index (LRI), ensuring accurate quantification without errors.

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