Hydrogen as Carrier Gas: A Reliable GC-MS Method for the Qualitative Analysis of Essential Oils

Unveiling the Composition: Achieving Accurate Qualitative Analysis of Essential Oils

Filippo Alibrando[1], Giuseppe Micalizzi[2], Luigi Mondello[1,2]

[1]Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

Abstract

This research is focused on the development of a gas chromatography-mass spectrometry (GC-MS) method for the analysis of essential oils by using hydrogen (H_2) as carrier gas. Helium (He) is conventionally used as carrier gas in GC-MS analysis due to its chemical properties such as inertia that yields optimal chromatography while minimizing undesirable reactions. However, alternative carrier gases such as H_2 have been recently considered due to the He shortage or its slow supply. To explore the separative performance, a lemon (*Citrus Limon L.*) essential oil was analyzed by GC-MS. The optimized method allowed the separation and identification of 55 volatile compounds including monoterpenes, sesquiterpenes, and oxygenated derivatives in comparable He-based analysis times. All components were identified by means of a dedicated mass spectral library combining mass spectra and linear retention index (LRI) values.

1. Introduction

The typical and unique smell of the essential oils generates a particular interest around the global flavor and fragrance market. Their composition contemplates a very complex mixture of monoterpene and sesquiterpene hydrocarbons, oxygenated derivatives, and aliphatic oxygenated compounds.[1] Just for their economic relevance, their characterization is very important to avoid authenticity fraud and to verify purity and safety. Gas chromatography coupled to mass spectrometry (GC-MS) is the most used analytical technique for the identification of terpenes in essential oils.[1] Such a technique guarantees high suitability in the identification of the unknown compound by means of mass spectral database. However, the technique is not able to distinguish isomers or compounds with similar chemical structures due to their spectral similarities, a drawback often observed in several fields including essential oil analyses.[2] For this reason, the identification process is also performed using relative retention criteria like linear retention index (LRI) values. Helium (He) is conventionally used as GC-MS carrier gas due to its chemical properties such as inertia that yields optimal chromatography while minimizing undesirable reactions. However, helium shortage or its slow supply has led to investigations of hydrogen (H_2) as alternative carrier gases for GC-MS analyses. Literature data indicate that H_2 can create some drawbacks such as activation of the GC injector liner or alteration of components thought interaction with the ion source metallic surface.[4] In general, the attitude of the analysts to accept and replace the well-established analytical procedures with rapid and cost-effective ones takes a long time and prevents their usage despite the neat gain in term of cost per analysis. H_2 is known to have the highest optimal linear velocity, thus rapid analysis times can be obtained. In fact, its optimum linear velocity is around 60 cm/s.[3] Also, H_2 respects the principles of the “green chemistry”, because its production does not contribute to environmental pollution, with an easier availability and cost significantly lower than the helium one.[5]

This research explores the performance of H_2 as GC-MS carrier gas for the analysis of essential oils. For this purpose, a lemon (*Citrus Limon L.*) essential oil was analyzed. The identification process was carried out using a mass spectral database containing LRIs used conventionally in He-based GC-MS analysis.

2. Experimental

2.1 Samples, chemicals, and sample preparation

A lemon (*Citrus Limon L.*) essential oil was kindly supplied by "Simone Gatto S.r.l." company (San Pier Niceto, Italy). 50 µL of essential oil were diluted in 950 µL of n-heptane (dil. 1:20). A C7-C30 saturated alkanes (1000 µg/mL) standard mixture in n-hexane was utilized for determining LRIs.

2.2 GC-MS analysis of the lemon essential oil

GC-MS Parameters	
Instrument:	GCMS-QP2020 NX (Shimadzu, Europa, Germany) equipped with a split-splitless injector and an AOC-20i autosampler
Column:	SLB®-5ms 30 m × 0.25 mm ID, 0.25 µm (**28471-U**)
Oven:	50 °C to 200 °C at 3 °C/min.
Injector temperature:	280 °C
Carrier gas:	H_2 at 60 cm/s of linear velocity (constant)
Initial inlet pressure:	14.1 KPa
Detector:	MDS
MS Conditions	
Ion source temperature:	220 °C
Interface temperature:	250 °C
Signal acquisition:	Scan mode with an event time of 0.2 sec, and a mass range of 40-660 amu

Data collection and data processing was carried out by using GCMS solution software (vers. 4.41, Shimadzu). The identification of compounds was performed using two different identification criteria: mass spectral similarity (≥ 85%) and LRI tolerance window (± 10 units).

3. Results and Discussion

3.1 GC-MS analysis of the lemon essential oil

GC-MS chromatogram of the lemon essential oil is shown in **Figure 1**. A total of 55 compounds, including monoterpene, sesquiterpene, and oxygenated derivatives were identified (**Table 1**). All components were eluted in about 37 min, in accordance with analysis times (ca. 45 min) of lemon essential oils obtained using He as carrier gas in GC-MS analyses.[6] However, a neat gain in term of cost per analyses was registered considering that H_2 gas was produced in laboratory by using generators based on the electrolysis, a process that separates water into hydrogen and oxygen. GC-MS is the most used technique for the qualitative screening of real complex matrices including essential oils. The identification of volatile substances is based on the usage of mass spectral database. However, isomer substances or components with similar chemical structures have identical fragmentation patters, resulting identical mass spectra. In that case, the identification of such molecules is impossible. To avoid mistaken peak assignment, the use of LRI in combination with MS spectra matching is strongly recommended. Thus, an accurate identification process requires the simultaneous searching of the structural information (MS similarity) and relative retention data (LRI correspondence) to confirm the identity of unknown compounds.[1]

In this research, the peak assignment was carried out by using FFNSC 4.0 library, a powerful tool which combines spectral and retention data. Such database was conventionally employed in He-based GC-MS analyses and no information about the identification power were available when alternative carrier gases were utilized.

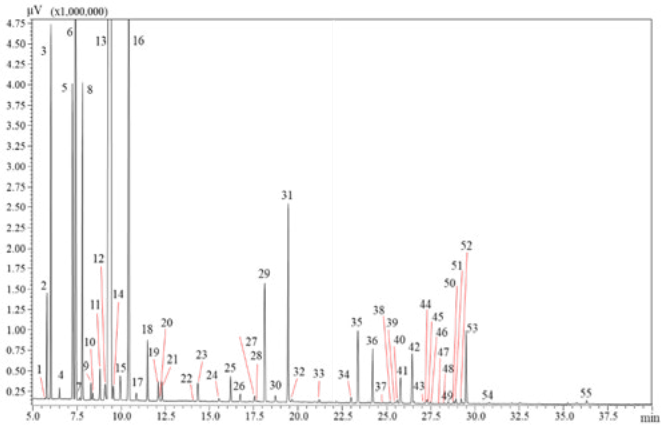

Figure 1. GC-MS chromatogram of the lemon essential oil obtained using H_2 as carrier gas (see **Table 1** for peak assignment).

Consequently, the herein proposed research was also focused on the exploitation of the database using H_2 as GC-MS carrier gas. As shown in **Table 1**, all compounds were identified with high values of spectral similarity indicating absolute matching between experimental and reference fragmentation patterns. Most compounds showed a spectral similarity of more than 90%. Absolute correspondence was also registered between experimental and reference LRI. The obtained terpenes profile of the lemon essential was in accordance with the data reported in literature.[6]

Table 1. Identification of the terpene compounds in lemon essential oil.

Abbreviation: MS Sim. represents mass spectral similarity; LRI ref: reference linear retention index; LRI exp: experimental linear retention index

ID	Name	Class	MS Sim.	LRI ref.	LRI exp.
1	Tricyclene	Monoterpene	89	923	921
2	α-Thujene	Monoterpene	98	927	924
3	α-Pinene	Monoterpene	97	933	931
4	Camphene	Monoterpene	90	950	947
5	Sabinene	Monoterpene	98	972	970
6	β-Pinene	Monoterpene	96	978	976
7	6-methyl-Hept-5-en-2-one	Ketone	95	986	983
8	Myrcene	Monoterpene	97	991	988
9	n-Octanal	Aldehyde	96	1006	1003
10	α-Phellandrene	Monoterpene	96	1007	1005
11	α-Terpinene	Monoterpene	97	1018	1016
12	p-Cymene	Monoterpene	97	1025	1023
13	Limonene	Monoterpene	97	1030	1031
14	(Z)-, β-Ocimene	Monoterpene	91	1035	1035
15	(E)-, β-Ocimene	Monoterpene	97	1046	1045
16	γ-Terpinene	Monoterpene	96	1058	1057
17	(Z)-Sabinene hydrate	Alcohol	91	1069	1068
18	Terpinolene	Monoterpene	97	1086	1084
19	Linalool	Alcohol	96	1101	1099
20	(E)-Sabinene hydrate	Alcohol	90	1099	1100
21	n-Nonanal	Aldehyde	96	1107	1104
22	Camphor	Alcohol	89	1149	1144
23	Citronellal	Aldehyde	97	1152	1150
24	Terpinen-4-ol	Alcohol	93	1184	1178
25	α-Terpineol	Alcohol	97	1195	1193
26	n-Decanal	Aldehyde	96	1208	1205
27	Nerol	Alcohol	96	1229	1224
28	Citronellol	Alcohol	93	1232	1227
29	Neral	Aldehyde	97	1238	1237
30	Geraniol	Alcohol	96	1255	1250
31	Geranial	Aldehyde	97	1268	1267
32	Perillaldehyde	Aldehyde	93	1278	1272
33	n-Undecanal	Aldehyde	93	1309	1307
34	Citronellyl acetate	Ester	94	1350	1349
35	Neryl acetate	Ester	97	1361	1357
36	(Geranyl acetate	Ester	98	1380	1377
37	Tetradec-1-ene	Alkene	90	1392	1391
38	n-Tetradecane	Alkane	90	1400	1400
39	n-Dodecanal	Aldehyde	89	1410	1408
40	α-, (Z)-Bergamotene	Sesquiterpene	92	1416	1410
41	(E)-Caryophyllene	Sesquiterpene	95	1424	1414
42	α-, (E)-Bergamotene	Sesquiterpene	97	1432	1430
43	Neryl propionate	Ester	89	1448	1447
44	α-Humulene	Sesquiterpene	93	1454	1451
45	(E)-, β-Farnesene	Sesquiterpene	91	1452	1451
46	β-Santalene	Sesquiterpene	94	1459	1456
47	Geranyl propanoate	Ester	89	1471	1468
48	γ-Curcumene	Sesquiterpene	89	1482	1475
49	β-,(E)-Bergamotene	Sesquiterpene	92	1483	1480
50	Valencene	Sesquiterpene	92	1492	1488
51	Bicyclogermacrene	Sesquiterpene	96	1497	1491
52	(Z)-, α-Bisabolene	Sesquiterpene	96	1503	1498
53	β-Bisabolene	Sesquiterpene	96	1508	1505
54	(E)-, α-Bisabolene	Sesquiterpene	89	1540	1538
55	α-Bisabolol	Alcohol	95	1688	1684

4. Conclusion

A GC-MS method based on the use of H_2 as carrier gas was optimized for the identification of the volatile substances in essential oils. For this purpose, a lemon essential oil was analyzed. 55 terpene compounds, including monoterpenes, sesquiterpenes, and oxygenate derivatives (aldehydes, ketones, alcohols, and esters) were identified by using two different identification criteria: mass spectral similarity and LRI correspondence. All components were eluted in 37 min, in accordance with analysis times obtained using He as a GC-MS carrier gas. A neat gain in term of cost per analyses was highlighted considering that H_2 gas was produced by using generators. Finally, carrier gas switching to H_2 did not necessitate to adjust or to modify mass spectral database containing MS spectra and LRI values. In fact, absolute correspondence between experimental and reference data were obtained. However, it must be underlined that safety issues should be well reviewed to ensure safe operations.

Summary

- The GC-MS method using hydrogen as a carrier gas offers a reliable and comparable alternative to helium for qualitative analysis of essential oils.
- The method successfully identified 55 volatile compounds in lemon essential oil, including monoterpenes, sesquiterpenes, and oxygenated derivatives, using a mass spectral library and linear retention index (LRI) values.
- Hydrogen as a carrier gas provides potential cost savings and aligns with the principles of green chemistry, without requiring adjustment of the mass spectral database.

For further information on sustainable products and solutions visit
SigmaAldrich.com/sustainable-chemistry

References

1. Trovato E, Micalizzi G, Dugo P, Utczás M, Mondello L. Use of linear retention indices in GC-MS libraries for essential oil analysis. In: Handbook of Essential Oils. Third edition. CRC Press; 2020. p. 229–251. DOI:10.1201/9781351246460-8
2. Zellner BD, Bicchi C, Dugo P, Rubiolo P, Dugo G, Mondello L. Linear retention indices in gas chromatographic analysis: a review. Flavour and fragrance journal. 2008;23(5):297–314. DOI:10.1002/ffj.1887
3. Heseltine J. Hydrogen as a carrier gas for GC and GC–MS. Chromatography Online. 2010 Jan 1, https://www.chromatographyonline.com/view/hydrogen-carrier-gas-gc-and-gc-ms
4. Margolin Eren KJ, Prest HF, Amirav A. Nitrogen and hydrogen as carrier and make–up gases for GC–MS with Cold EI. Journal of mass spectrometry. 2022;57:4830. DOI:10.1002/jms.4830
5. Bartram RJ, Froehlich P. Considerations on switching from helium to hydrogen. Chromatography Online. 2010 Oct 1, https://www.chromatographyonline.com/view/considerations-switching-helium-hydrogen.
6. Dugo P, Ragonese C, Russo M, Sciarrone D, Santi L, Cotroneo A, Mondello L. Sicilian lemon oil: Composition of volatile and oxygen heterocyclic fractions and enantiomeric distribution of volatile components. Journal of separation science. 2010;33(21):3374–3385. DOI:10.1002/jssc.201000578

Featured & Related Products

Description	Cat. No.
GC Column	
SLB®-5ms 30 m x 0.25 mm, 0.25 µm	**28471-U**
Reference Material	
C7 - C30 Saturated Alkanes, certified reference material, 1000 µg/mL each component in hexane, ampule of 1 mL	**49451-U**
Solvents	
Heptane, ReagentPlus®, 99%	**H2198**
Hexane, ReagentPlus®, ≥99%	**139386**
Acetone, suitable for HPLC, ≥99.8%	**34850-M**
Related Products	
C7 - C40 Saturated Alkanes Standard, certified reference material, 1000 µg/mL each component in hexane, ampule of 1 mL	**49452-U**
C4 - C24 Even Carbon Saturated FAMEs, 1000 µg/mL each component in hexane, analytical standard, ampule of 1 mL	**49453-U**
Fatty Acid Ethyl Esters (FAEES), C4 - C24 Even Carbon Saturated, certified reference material, 1000 µg/mL each component in hexane, ampule of 1 mL	**49454-U**
Terpene Mix A, certified reference material, 2000 µg/mL each component in methanol, ampule of 1 mL	**CRM40755**
Terpene Mix B, certified reference material, 2000 µg/mL each component in methanol, ampule of 1 mL	**CRM40937**

See more about GC columns & accessories at
SigmaAldrich.com/GC

Find more standards and reference materials under
SigmaAldrich.com/standards

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
Technical Service: **SigmaAldrich.com/techservice**

SigmaAldrich.com

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®

MK_AN13440EN Ver. 1.0
54491
04/2024