Application Note

Instrument: Pegasus® BT

FAST GC-TOFMS and Hydrogen Carrier Gas: An Enhanced Solution for the Analysis of Citrus Essential Oils

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Key Words:Fast GC, Hydrogen, TOFMS, Flavor and Fragrance, Essential Oil, Citrus, Quality Control

Introduction

Citrus essential oils (Citrus EOs) are cold extracted from the peels of citrus fruit such as sweet orange, mandarin, lemon, grapefruit, lime, bitter orange, bergamot, clementine, etc. using purely mechanical systems. The volatile fraction of these oils ranges between 85 to 99% of the whole extracted oil.[1] This fraction mostly consists of mono-sesquiterpenes and their oxygenated derivatives, such as aldehydes, esters, ethers, and oxides. The ratio between characteristic molecules and/or the presence of specific "markers" is normally used to evaluate both the quality of a Citrus EO and its presence in complex mixtures.

Citrus EOs are used in many different fields such as cosmetics, cleaning products, food, and pharmaceuticals, although the largest amount is used in the fragrance industry. In all cases, they are often in direct contact with humans (i.e., ingestion, skin, pills, etc.) and this requires a full characterization of all the constituents to assess their quality, authenticity, and beyond that, to exclude the presence of harmful substances.

The qualitative characterization of the volatile fraction of EOs is generally attained by gas chromatography-mass spectrometry (GC-MS). The methods developed for this purpose typically use long GC capillary columns and apply slow oven ramp rates which translates into analysis times that are greater than one hour in most cases. In addition, most of these methods use helium (He) as a carrier gas for the GC-MS instrumentation due to manufacturer requirements and/or poor acceptance of hydrogen (H_2) as a GC-MS carrier gas. All these factors contribute to high economical costs and also limit laboratory throughput.

This application note describes the development of a rapid and robust H_2-supplied GC method coupled to LECO's *Pegasus* BT time-of-flight mass spectrometer (GC-TOFMS), for a fast and reliable analysis of Citrus EO samples. Specifically, a Citrus EO mix ("Citrus mix"), composed of multiple individual Citrus EOs was analyzed with the objective to quickly determine/identify which individual Citrus EOs were originally blended for its constitution.

The method transfer approach illustrated in the Application Note 203-821-651 was applied to this sample. A total of five transfer and optimization steps were conducted.

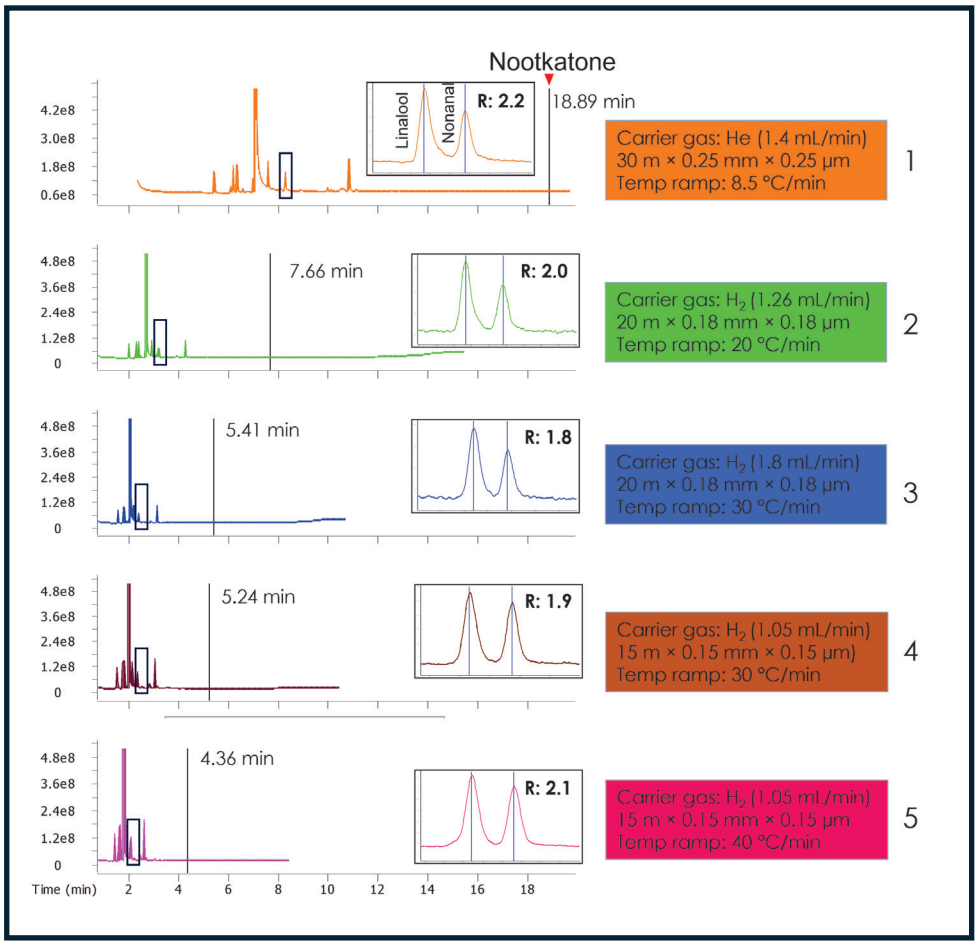

Figure 1: GC-TOFMS chromatograms of each method transfer step. The retention time of nootkatone is indicated in every chromatogram as a reference for the last eluting component. In the inserts, the resolution values, automatically calculated by ChromaTOF® brand software, are listed for linalool and nonanal under the different GC conditions applied.

Experimental

The Citrus mix was purchased at a local store, and it was sold as a multipurpose product (e.g. aromatherapy, air freshener, etc.). The sample was diluted with a factor 200:1 in hexane. An n-Alkane standard mixture (C7-C30) was diluted to 10 mg/L in hexane and analyzed under the same conditions for calculation of linear retention indices (RIs). Table 1 provides the instrumental parameters applied for the Citrus mix analyses. The table displays the initial GC parameters for He- and the final optimized H_2-based method.

Table 1: Analytical parameters for the Citrus mix analysis for initial He- and the final optimized H_2-based method.

	Injector	
Split Mode	1 µl (200:1 citrus mix; 10:1 alkanes) at 280 °C	
GC	**Agilent 7890 (He standard)**	**Agilent 7890 (H_2 optimized)**
Carrier Gas	He 1.4 mL/min	H_2 1.05 mL/min
Column	Rxi-5Sil MS 30 m x 0.25 mm i.d. x 0.25 µm coating	Rxi-5Sil MS 15 m x 0.15 mm i.d. x 0.15 µm coating
Oven Program	45 °C; ramp: 8.5 °C/min to 250 °C	45 °C; ramp: 40 °C/min to 250 °C
Transfer Line	280 °C	280 °C
MS	**LECO *Pegasus* BT**	
Ion Source Temp	250 °C	
Mass Range	40 – 400 m/z	
Acquisition Rate	10 spectra/s	40 spectra/s

Method transfer was done by analyzing the sample with multiple GC conditions. Figure 1 shows representative chromatograms of each method transfer step. In total, five transfer and optimization steps were conducted, each labeled with a different color and number (1-5) in Figure 1. The last eluting compound of interest was nootkatone (CAS: 4674-50-4) and it was used as reference to determine the total run time for the different chromatographic conditions.

Results

Figure 1 shows the chromatograms for each method transfer step and the time of the last eluting compound of interest. In the initial He-based method, nootkatone was eluting at 18.89 minutes. As expected, the total run time per analysis decreased drastically along the transfer and optimization steps. Eventually, the final H_2-based method resulted in an elution time for nootkatone of 4.36 minutes, which is an overall time saving factor of approximately 4.3. This reduction in analysis time did not result in a signification reduction of chromatographic resolution. Linalool (left peak, CAS: 78-70-6) and nonanal (right peak, CAS: 124-19-6) are highlighted in the zoomed-in area on the right top of every chromatogram. The resolution (Rs) between the two peaks was automatically calculated in *ChromaTOF* software and is provided for all experimental conditions. Taking a closer look into the area of these two important, but closely eluting compounds reveals that the decrease of resolution, when changing from the initial (He) to the final (H_2) method, is only about 10%, while the analysis time reduced by ~77% (from 18.89 minutes to 4.36 minutes).

A tailored data processing method that incorporates LECO's Non-Target Deconvolution® algorithm (NTD®) and automated RI calculation was established to identify as many analytes as possible. The deconvoluted spectra were searched against the NIST mass spectral database (NIST 17) including the RI information on the same column type. The *ChromaTOF* software features automated library hit filtering according to the library's RI data providing increased confidence in compound identification. The RI criteria for hit filtration are user defined, allowing for tailored data processing. This feature revealed to be crucial for a correct identification of EOs constituents, as they mainly consist of terpene isomers with similar mass spectral fragmentation patterns.

Table 2 reports the key components identified in the Citrus mix sample along with their retention time (R.T.), experimental RI (RIexp), library RI (RILibrary), and library score based on the final H_2 optimized data (Experiment 5). Overall, high quality mass spectral information (i.e., high library scores) as well as good agreement between RIexp and RILibrary were obtained for the all the identified components. In total, 57 components were identified with a library similarity score higher than 750. In this respect an average library score of 860/1000 was obtained.

Table 2: List of key Citrus EOs components identified from the optimized H_2 method (Experiment 5) including the name, library retention index ($RI_{Library}$), experimental retention index (RI_{Exp}), library scores, and CAS number.

Peak #	Name	R.T. (min)	$RI_{(exp)}$	$RI_{(Library)}$	Similarity	CAS No
1	3-Hexanone	0.852	773	784 ± 7	838	589-38-8
2	2-Hexanone	0.867	777	790 ± 3	808	591-78-6
3	Octane	0.902	787	800	834	111-65-9
4	Nonane	1.270	893	900	898	111-84-2
5	α-Thujene	1.380	922	929 ± 2	927	2867-05-2
6	α-Pinene	1.413	931	937 ± 3	939	80-56-8
7	α-Fenchene	1.474	947	950 ± 3	822	471-84-1
8	Camphene	1.482	949	952 ± 2	888	79-92-5
9	1-Heptanol	1.546	965	970 ± 2	862	111-70-6
10	Bois de Rose oxide	1.557	968	972 ± 3	859	7392-19-0
11	Sabinene	1.569	971	974 ± 2	902	3387-41-5
12	β-Pinene	1.593	977	979 ± 2	951	18172-67-3
13	β-Myrcene	1.627	986	991 ± 2	945	123-35-3
14	Octanal	1.680	1000	1003 ± 2	916	124-13-0
15	p-Mentha-1(7),8-diene	1.694	1004	1004 ± 3	907	499-97-8
16	α-Phellandrene	1.703	1006	1005 ± 2	901	99-83-2
17	δ-3-Carene	1.715	1009	1011 ± 2	917	13466-78-9
18	1,4-Cineole	1.737	1015	1016 ± 2	893	470-67-7
19	α-Terpinen	1.746	1017	1017 ± 2	855	99-86-5
20	o-Cymene	1.776	1025	1025 ± 2	921	99-87-6
21	Limonene	1.801	1031	1030 ± 2	855	138-86-3
22	p-Cymene	1.806	1032	1025 ± 2	766	99-87-6
23	trans-β-Ocimene	1.855	1045	1049 ± 2	867	3779-61-1
24	γ-Terpinene	1.909	1059	1060 ± 3	896	99-85-4
25	1-Octanol	1.945	1068	1071 ± 3	906	111-87-5
26	Sabinene hydrate, cis	1.958	1071	1070 ± 4	788	15537-55-0
27	Benzenemethanol, α,4-dimethyl-	2.003	1083	-	770	536-50-5
28	Terpinolene	2.019	1087	1088 ± 2	912	586-62-9
29	p-Cymenene	2.034	1091	1090 ± 2	845	1195-32-0
30	Linalool	2.062	1098	1099 ± 2	844	78-70-6
31	Nonanal	2.080	1103	1104 ± 2	857	124-19-6
32	Fenchol	2.152	1122	1113 ± 4	805	1632-73-1
33	Limonene oxide, cis-	2.202	1136	1134 ± 4	808	13837-75-7
34	Terpinen-1-ol	2.212	1138	1137 ± 4	827	586-82-3
35	Limonene oxide, trans-	2.217	1140	1134 ± 4	838	13837-75-7
36	Isopulegol	2.263	1152	1146 ± 3	867	89-79-2
37	Octanoic acid	2.299	1161	1180 ± 7	869	124-07-2
38	endo-Borneol	2.357	1177	1167 ± 2	830	507-70-0
39	Terpinen-4-ol	2.386	1185	1177 ± 2	793	562-74-3
40	α-Terpineol	2.436	1198	1189 ± 2	924	98-55-5
41	Decanal	2.463	1205	1206 ± 2	896	112-31-2
42	Linalyl acetate	2.618	1249	1257 ± 3	852	115-95-7
43	Perillaldehyde	2.731	1281	1272 ± 4	825	2111-75-3
44	Neryl acetate	2.992	1357	1364 ± 3	815	141-12-8
45	Geranyl acetate	3.057	1376	1382 ± 3	829	105-87-3
46	Copaene	3.085	1385	1376 ± 2	897	3856-25-5
47	β-Cubebene	3.121	1395	1389 ± 2	759	13744-15-5
48	Dodecanal	3.167	1409	1409 ± 4	912	112-54-9
49	Caryophyllene	3.236	1431	1419 ± 3	933	87-44-5
50	trans-α-Bergamotene	3.263	1439	1435 ± 3	913	13474-59-4
51	(E)-β-Farnesene	3.309	1454	1457 ± 2	803	18794-84-8
52	Germacrene D	3.429	1492	1481 ± 3	761	23986-74-5
53	Valencene	3.465	1503	1492 ± 3	815	4630-07-3
54	β-Bisabolene	3.494	1512	1509 ± 3	898	495-61-4
55	δ-Cadinene	3.536	1526	1524 ± 2	886	483-76-1
56	α-Sinensal	4.190	1753	1752 ± 4	754	17909-77-2
57	Nootkatone	4.364	1817	1808 ± 10	825	4674-50-4

Figure 2 shows a comparison between the MS spectra of linalyl acetate (peak #42) recorded from the He and H_2 optimized runs (i.e., 1 and 5). As can be seen, both experimental spectra show similar fragmentation patterns compared to the NIST library hit, regardless of the carrier gas employed.

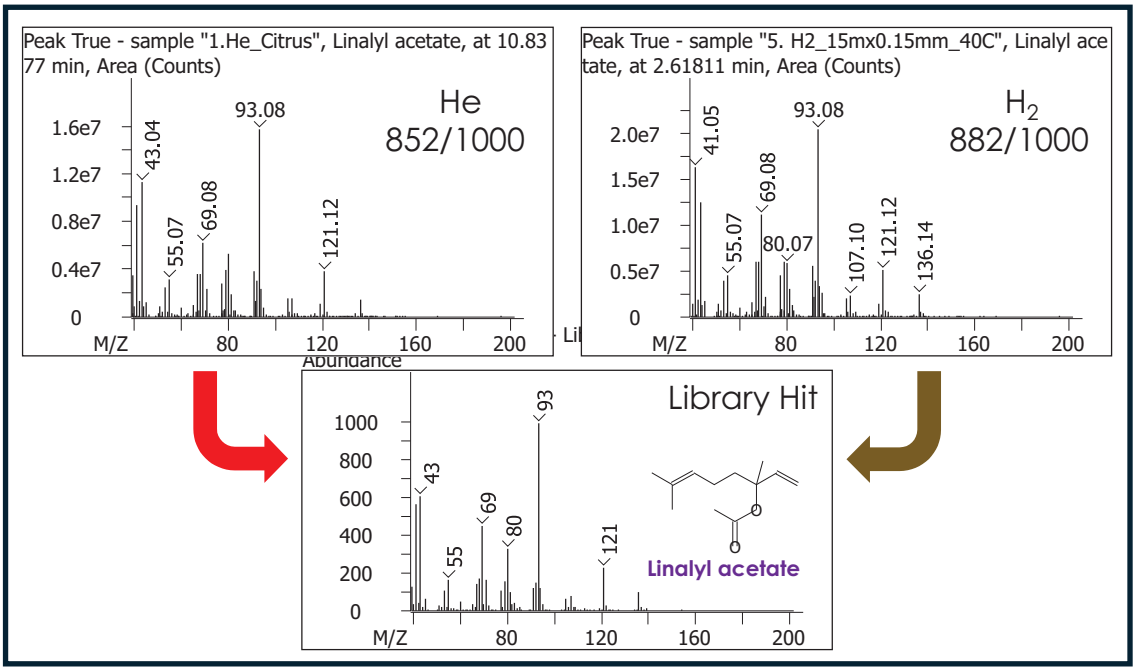

Figure 2: Linalyl acetate MS spectra obtained for the optimized He GC-TOFMS methods (top left), optimized H_2 GC-TOFMS method (top right), and the NIST library spectrum (bottom center).

In addition, when using narrow bore columns (i.e. 15 m x 0.15 mm ID, Experiment 5), the acquisition rate of the mass spectrometer becomes an important factor to optimize in order to obtain enough data points across a chromatographic peak. We selected an acquisition rate of 40 spectra/s for the GC-TOFMS analysis. This provided enough data points for proper peak construction and was also optimal for LECO's *ChromaTOF* deconvolution algorithm, allowing trace peaks coeluting with large base peaks to be successfully determined. As an example, the deconvolution of Limonene (peak #21), a predominant and slightly overloaded peak in the Citrus mix sample, and p-cymene (peak #22) is shown in Figure 3. p-cymene was efficiently deconvoluted and its library score is well within the range of positive identifications.

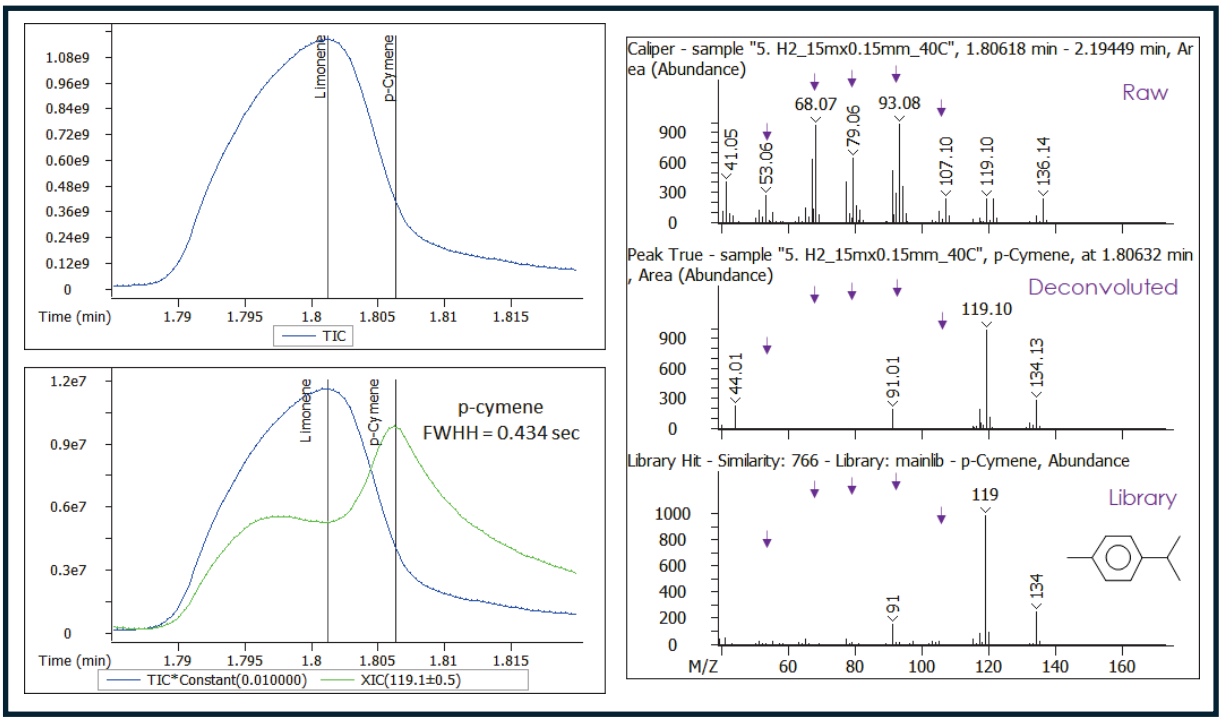

Figure 3. Example of deconvolution of Limonene (peak #21) and p-cymene (peak #22); p-cymene's Caliper spectrum (top) clearly shows the presence of multiple m/z fragments belonging to Limonene and hence removed from the Peak True spectrum (middle).

Finally, the H_2 optimized data were further interrogated with the goal of gathering as much information as possible regarding the individual Citrus EOs present in the Citrus mix. The presence or absence of specific compounds provided good insight to which Citrus EOs were likely used in the Citrus mix. As an example, linalyl acetate (peak #42) was identified with a good library similarity score (852/1000) and RI difference (Δ = 8): this component is very characteristic of bergamot EOs where it is present in large amounts. Another component of interest is α-sinensal (peak #56), a component very characteristic for mandarin EOs, although it can be also found at trace levels in sweet orange, tangerine, and clementine EOs. Furthermore, the Citrus mix also contained a relatively high amount of δ-3-carene (peak #17) and traces of valencene (peak #53), which suggest the presence of sweet orange. The presence of mandarin EO can be excluded due to the absence of methyl-N-methyl anthranilate, another typical component of such EO. The latest eluting component of interest was the nootkatone (peak #57) which is mainly present in grapefruit EOs, but it can also be found in other EOS such as bitter orange, lemon, and bergamot.[2]

Conclusion

The method transfer from a He- to a H_2-supplied LECO *Pegasus* BT GC-TOFMS system was easily conducted in a few steps. The transition to H_2 provides a tremendous decrease of analysis time translating directly in reduction of analysis cost, all whilst resolution, spectral quality, and sensitivity are maintained or even improved, enabling an efficient determination of the compounds of interest. For this application, the use of H_2 as carrier gas allowed analysis time to decrease by a factor of 4.3 while maintaining chromatographic resolution and data quality. Although the Citrus EOs sample presented a certain degree of complexity, thanks to LECO's deconvolution and the spectral quality attained, it was possible to identify most of the initial EOs mixed to obtain the final blend. Thus, this approach can be considered powerful and suitable for quality control and/or fingerprinting of EOs in laboratories where throughput and quality of results are the key point of success.

References

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[2]G. Dugo, L. Mondello. Citrus Oils, CRC Press: Boca Raton, Florida, 2011; 1–161.

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