Application Note

Instrument: Pegasus® BT

A Rapid, Robust and Sensitive Analysis of Tea Tree Essential Oil Quality by GC-TOFMS with Hydrogen as Carrier Gas

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Introduction

Essential oils and/or their components are well-known for their aromatic properties and are commonly used in many applications e.g., perfumery, food and agricultural, as well as pharmaceutical and cosmetic industries.[1,2,3] This wide range of application areas can be explained by the various activities which are associated with essential oils; the long list of positive attributes includes antibacterial, antifungal, anti-inflammatory, antimicrobial, antioxidant, and antiviral effects, among others.[4,5] In addition, essential oils are often reported as relatively safe, natural, and effective agents, resulting in a broad consumer acceptance. The production process is based on hydro-distillation of plant material or plant parts, or cold-pressing for citrus oils. Even though many constituents are widely prevalent in nature, often it is the ratio of specific components which define the physical and physiological signature, and therefore the quality, of a particular essential oil. Such an example is tea tree oil (TTO), which is widely used as an important ingredient in a variety of consumer products, including skin and hair care lotions, cleaning formulations, and fragrances. The standard, ISO 4730, specifies selected components to be part of TTO for a variety of reasons, including authenticity, provenance verification, and biological activity. For example, the incorporation of the minor components globulol and viridiflorol is potentially helpful, as it may render the formulation of artificial oil from individual components difficult or economically untenable.

The shortage of helium and its related increase in costs is urging laboratories to find suitable alternatives. This application note describes a rapid and robust gas chromatographic method coupled to LECO's Pegasus® BT time-of-flight mass spectrometer (GC-TOFMS) for the high-quality analysis of components in TTO. For this purpose, a method previously developed for a helium (He)-supplied GC-TOFMS *Pegasus* BT was translated and optimized for hydrogen (H_2) with the help of the EZGC Method Translator and Flow Calculator (https://ez.restek.com/ezgc-mtfc). In total, five translation and optimization steps were conducted. Here, the ease of optimum chromatographic method transfer in switching from He to H_2 are reported, in addition to evidence regarding the overall quality of H_2-based methods, in terms of mass spectral quality, robustness and sensitivity.

Figure 1 shows representative chromatograms of each method translation step. In total, five translation and optimization steps were conducted, each of them labelled with a different color. The last eluting compound of interest was globulol (CAS: 51371-47-2) and it was used as a reference point to compare the required run time for the different chromatographic conditions.

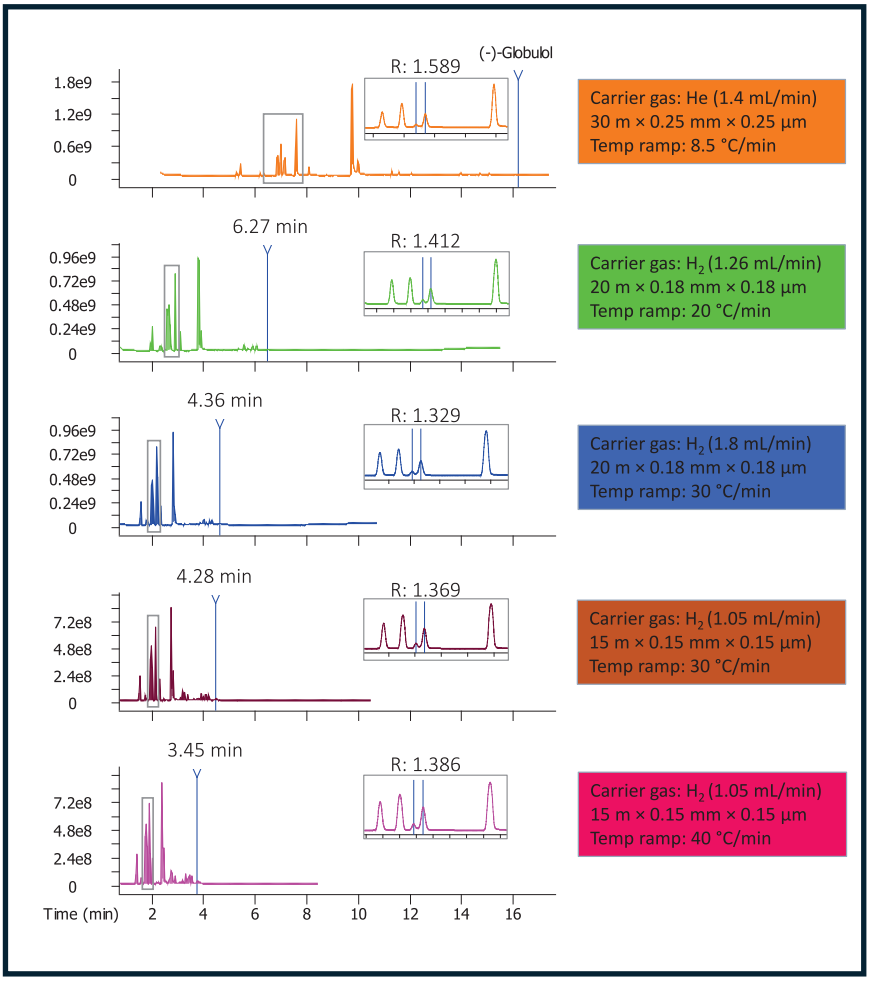

Figure 1: Chromatograms of each method translation step. As reference point for analysis speed, the elution time of globulol is indicated in every chromatogram.

Experimental

A sample of tea tree (*Melaleuca alternifolia*) essential oil was obtained commercially for the study. The TTO was diluted with a factor 100:1 in hexane. n-Alkane standards (C7-C30) were diluted to 10 ppm in hexane and analyzed under the same conditions for calculation of linear retention indices (RIs). Table 1 provides the instrumental parameters for the tee tree oil analyses. The table displays the initial gas chromatographic parameters for He and the final optimized H_2-based method.

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

	Injector	
Split Mode	1 µl (200:1 TTO; 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 330 °C	45 °C; ramp: 40 °C/min to 330 °C
Transfer Line	280 °C	280 °C
MS	LECO *Pegasus* BT	
Ion Source Temp	250 °C	
Mass Range	40–400 m/z	
Acquisition Rate	30 (spectra/s)	
Extraction Frequency	32 kHz	

Results and Discussion

Figure 1 shows the chromatograms for each method transfer and the time of the last eluting compound of interest. In the initial He-based method, globulol was eluting at 16.03 min. The run time per analysis decreases throughout the translation and optimization steps drastically. Eventually, the final H_2-based method resulted in an elution time for globulol of 3.54 min, which is an overall time saving factor of approximately five. Limonene (left peak, CAS: 138-86-3) and eucalyptol (right peak, CAS: 470-82-6) are highlighted in the zoomed-in area on the right top of every chromatogram. The resolution (R) of the two peaks was calculated automatically in the ChromaTOF® brand 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 12% while the analysis time reduced by ~78% (from 16.03 min to 3.45 min).

The tailored data processing for the detection of key terpenes was accomplished including the software-integrated automated retention index matching ameliorating compound identification. Table 2 reports key TTO terpenes (according to ISO 4730) with their respective retention indices (RIs) and library scores for the initial (He) and the final (H_2) method. High quality mass spectral information obtained with the two exemplarily displayed methods resulted in high library scores for all target compounds. Moreover, the translation from a "conventional" to "fast" GC-TOFMS method, as well documented in the application note, produced an increase in the overall sensitivity. An average increment of ~40% was observed for the final H_2 optimized Fast GC-TOFMS method.

Table 2: List of key terpenes with name, chemical formula, library retention index ($RI_{Library}$), experimental retention index (RI_{Exp}), library scores and signal-to-noise (S/N) for the initial (He) and the final (H_2) method.

			Initial (He)			Final (H_2)		
Name*	**Formula**	**$RI_{Library}$**	**RI_{Exp}**	**Score**	**S/N**	**RI_{Exp}**	**Score**	**S/N**
α-Thujene	$C_{10}H_{16}$	929±2	928.1	902	6191	922.4	934	10475
α-Pinene	$C_{10}H_{16}$	937±3	936.2	926	16930	930.8	946	32159
α-Terpinene	$C_{10}H_{16}$	1017±2	1019.2	832	14740	1016.8	879	21875
p-Cymene	$C_{10}H_{14}$	1025±2	1026.4	911	41980	1024.4	901	62295
Limonene	$C_{10}H_{16}$	1030±2	1031.6	932	4615	1029.6	940	9505
1,8-Cineole	$C_{10}H_{18}O$	1032±2	1035	907	5398	1033.3	922	9547
γ-Terpinene	$C_{10}H_{16}$	1060±2	1060.2	850	11652	1059.1	901	15357
Terpinolene	$C_{10}H_{16}$	1088±2	1087.9	920	6482	1087.1	920	8806
Terpinen-4-ol	$C_{10}H_{18}O$	1177±2	1183.7	845	26848	1185.4	756	31320
α-Terpineol	$C_{10}H_{18}O$	1189±2	1196.9	924	5813	1198	935	8190
Aromadendrene	$C_{15}H_{24}$	1440±1	1447.3	914	430	1450.1	937	1030
1,2,4-trihydroxymenthane	$C_{10}H_{20}O_3$	1487±1	1489.8	859	743	1492.7	789	1393
Ledene/viridiflorene	$C_{15}H_{24}$	1493±4	1498.8	893	942	1501.3	923	2363
α-Muurolene	$C_{15}H_{24}$	1499±3	1504.2	801	468	1504.5	868	817
α-Cadinene	$C_{15}H_{24}$	1524±2	1524.1	875	2662	1526.1	896	8168
Viridiflorol	$C_{15}H_{26}O$	1591±2	1571	858	92	1574	858	113
Globulol	$C_{15}H_{26}O$	1591±11	1594.8	899	269	1598	892	284
Average				*885*			*894*	

**All compounds from ISO 4730 plus 1,2,4-trihydroxymenthane and α-Muurolene.*

Figure 2 provides a more detailed evaluation of spectral quality. Despite the fact that viridiflorol was only present in low abundance, no negative impact on the spectral quality was observed when moving from He to H_2 as a carrier gas, as the library scores demonstrated. The library scores were 858/1000 for both the He and H_2 Fast GC-TOFMS method.

With regards to sensitivity, especially the detection of trace level compounds benefits from the transition from He to H_2 and conventional to fast chromatography. Figure 2 shows the deconvoluted mass spectrum and the NIST library match for viridiflorol (CAS: 552-02-3). The left side displays the results obtained with the initial method (He). The S/N in the He-based method (left side) was 92, whereas the final H_2-based method resulted in a S/N value of 132. The same trend was also observed for all other compounds in focus (see Table 2).

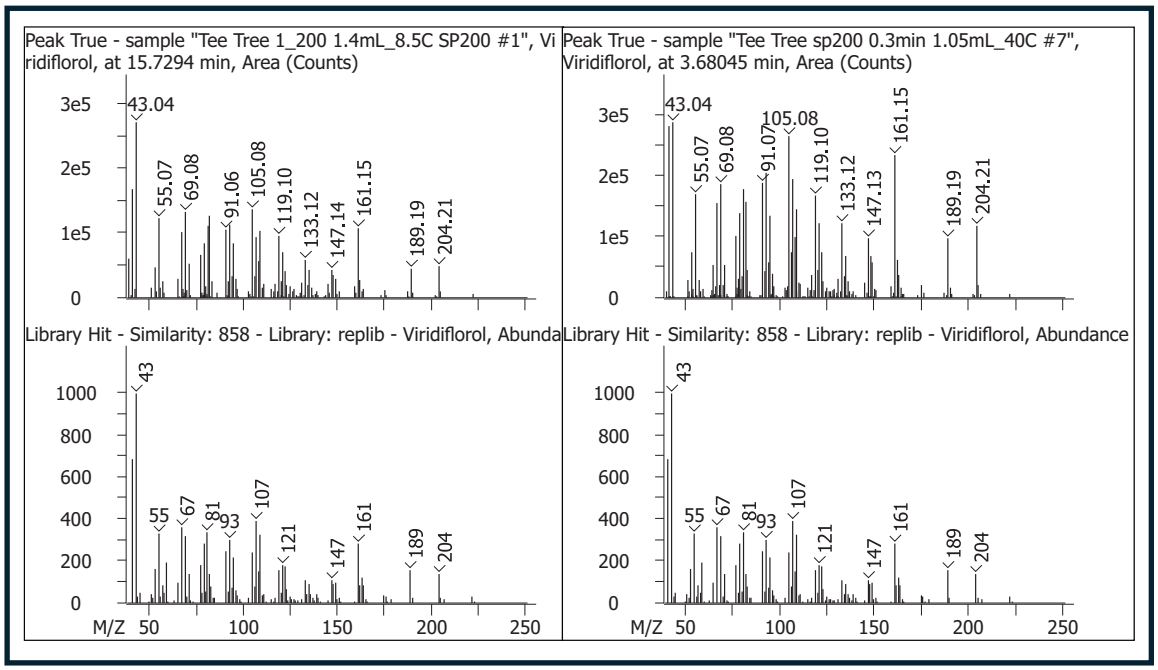

Figure 2: Deconvoluted mass spectrum (top) and NIST library mass spectrum (bottom) of viridiflorol (CAS: 552-02-3) for the initial (He, left) and the final (H_2, right) method.

The quality of essential oils such as TTO is often impacted by their stability during processing, or under various storage conditions over time. Therefore, reliable and fast analytical screening for key components and their degradation products is an important part of quality control: for example, finding the ratio of caryophyllene (CAS: 13877-93-5) and its oxidation product caryophyllene oxide (CAS: 1139-30-6) (see Figure 3).

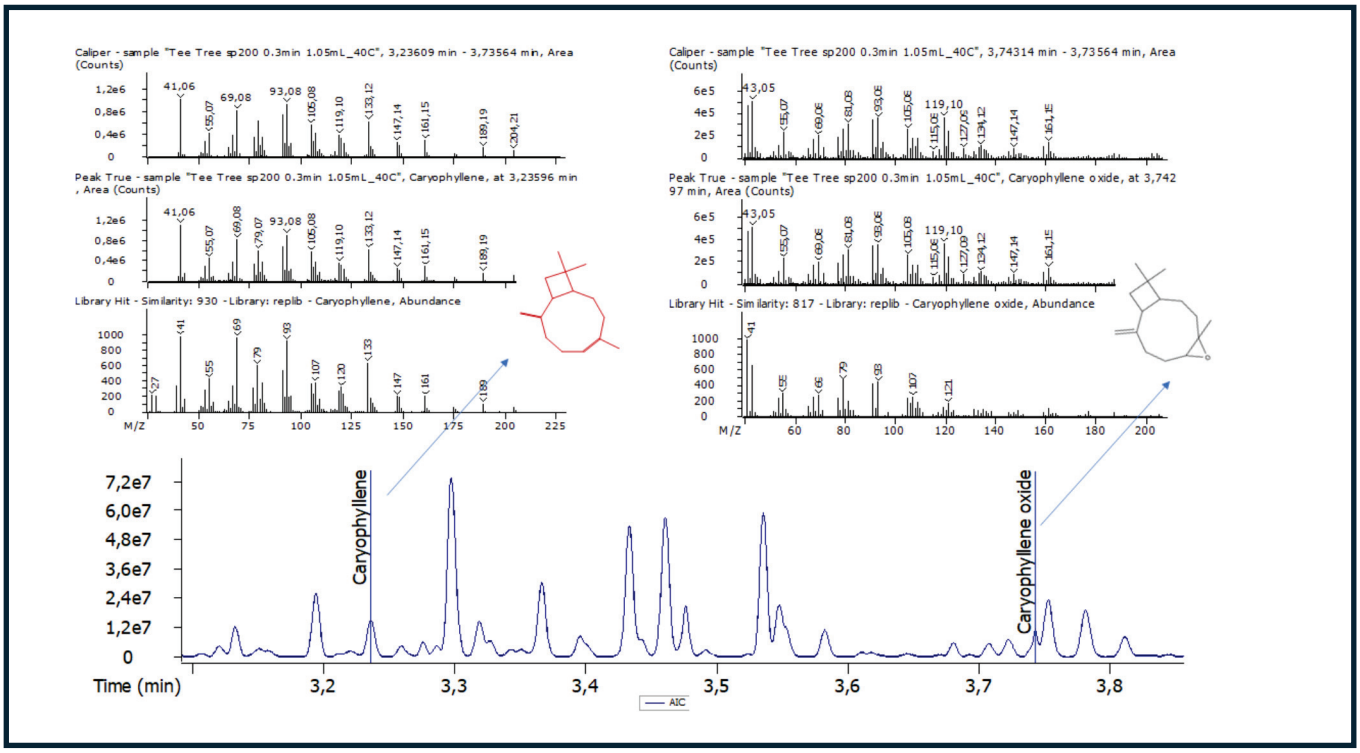

Figure 3: An example of fast screening for assessment of quality critical species using H_2 carrier gas optimized methodology and automated deconvolution. Monitoring degradation levels of key species via ratios to their by-products, such as with caryophyllene and caryophyllene oxide, is a key task, aided significantly by high-speed analysis and high confidence mass spectral library hit similarities.

Conclusion

The method transfer from a He to a H_2-supplied LECO *Pegasus* BT GC-TOFMS system can be easily conducted in a few steps without delay times due to baseline stabilization, ion source "acclimatization," and so on. The transition to H_2 results in a tremendous decrease of analysis time, translating directly into a reduction of analysis cost, while maintaining or increasing resolution, spectral quality, and sensitivity. This enables an efficient determination of compounds of interest. For this application, the use of H_2 as carrier gas allowed us to speed up analysis time by a factor of 5 while maintaining chromatographic resolution and without any loss in data quality. The identification relied on retention index matching and mass spectral comparison with the NIST mass spectral library, ultimately allowing for reliable identification of key terpenes in TTO. Thus, the presented approach is powerful and very suitable for quality control or fingerprinting of essential oils in laboratories where high throughput and quality of results are the most important factors.

References

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