Terpene Analysis by GC-VUV

A New Tool for GC Analysis of Terpenes in Flavors and Fragrances

APPLICATION NOTE

Introduction

Terpenes contribute heavily to the senses of smell and taste and are widely utilized in the flavor and fragrance, cosmetic, and pharmaceutical industries.[1,2] They are a class of organic compounds produced mostly by plants and are used as building blocks in many biosynthetic pathways by fungi, plants, and animals (for example, endogenously synthesized steroids are derived from the triterpene squalene). All terpenes are composed of multiple units of isoprene (C5H8), resulting in numerous isomeric configurations for each base molecular composition. The two most common classes of terpenes associated with odor and flavor are monoterpenes (two isoprene units) and sesquiterpenes (three isoprene units).

Terpene characterization has traditionally been performed through a combination of gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). Baseline resolution of analyte peaks is often needed for accurate quantification by either technique due to the isomeric nature of many terpenes. The burden of complete separation can lead to relatively long GC run times. Gas chromatography–vacuum ultraviolet spectroscopy (GC–VUV) can spectrally distinguish isomers and quantitatively deconvolve co-eluting peaks, allowing for a significant reduction in analysis time.

GC-VUV spectral data is inherently three dimensional (time, absorbance, wavelength) and specific to electronic structure. Nearly all compounds absorb in the VUV region of the electromagnetic spectrum with the exception of carrier gases hydrogen, helium, and argon. The VUV photons probe electronic transitions in virtually all chemical bonds including ground state to excited state $\sigma \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$, which cannot typically be accessed by traditional UV/Vis absorption spectroscopy.[3] The result is VUV spectral "fingerprints" that are specific to individual compound structure and can be readily identified by the VUV library. Unique VUV spectra enable closely related compounds such as structural isomers to be clearly differentiated. They can also be used to deconvolve co-eluting peaks with a high degree of accuracy. These combined characteristics enable significant reduction of GC run times through flow rate-enhanced chromatographic compression.

VUV spectroscopy follows the simple linear relationship between absorbance and concentration described by the Beer-Lambert Law. A measured VUV absorbance spectrum is the sum of the individual analyte spectra. An algorithm called time interval deconvolution (TID) algorithm was created that sums the total contribution of each analyte at every time interval within a specified time range, and the total contribution of each analyte is summed for a "total response" value.[4] This value can then be weighted with a relative response factor, density, and external calibration curve to yield a relative mass percentage, relative volume percentage, and absolute concentration, respectively. VUV Analyze™ software automates this process and deconvolves co-elution when it is encountered.

A GC–VUV and static headspace method was developed for fast terpene analysis that resulted in sub 9-min elution times for 21 terpenes.

VUV ANALYTICS

It has also been demonstrated that compositional analysis can be automated to provide relative quantities of terpenes in mixtures like essential oils.

Experimental

- Instrumentation and Standards

The GC–VUV and static headspace method used the following setup:

Detector: VUV Analytics VGA-100 GC detector

Gas Chromatograph: Agilent 6890 GC

Autosampler: GERSTEL Multi-Purpose Sampler (MPS)

Column: 30 m x 0.25 mm, 1.40-µm Rxi-624Sil MS (Restek)

The GC–VUV method for compositional analysis of essential oils utilized liquid injections and the setup noted below:

Detector: VUV Analytics VGA-100 GC detector

Gas Chromatograph: Agilent 6890 GC

Column: 30 m x 0.25 mm, 0.25-µm Rxi-1 MS (Restek)

Additional experimental conditions are detailed in subsequent paragraphs.

- Gas Chromatograph

The GC–VUV and static headspace method used an initial oven temperature of 60 °C (held 0.1 min), followed by a ramp at 23.8 °C/min to 300 °C. 2.5-mL injections were made into a 250 °C inlet with a 2.5:1 split ratio and a constant flow rate of 4 mL/min helium.

The method for compositional analysis of essential oils started at 70 °C (held of 0.1 min), then ramped at 8.5 °C/min to 250 °C. 1-µL injections entered a 250 °C inlet using a split ratio of 250:1 with a constant flow rate of 2 mL/min helium.

- Static Headspace Sampler

Vials were incubated at 80 °C and agitated for 10 min; a 2.5-mL aliquot of sample was extracted at 90 °C for injection into the GC system.

- VUV Detector

The GC–VUV and static headspace method held the transfer line and flow cell at 275°C, and the makeup gas pressure was set at 0.25 psi nitrogen. Spectral data were acquired at 4 spectra per second. The compositional analysis of essential oils utilized the same settings except for an acquisition rate of 10 spectra/sec.

Results and Discussion

- GC-VUV Analysis of Terpene Standards

Separation times for terpene analysis using GC–FID or GC–MS can take 30 min or more. The GC–VUV method developed for 21 terpene standards reduced the GC run time to approximately 10 min, with the last analyte eluting before 9 min (Figure 1). Several co-elutions were observed for the early-eluting monoterpenes, including β-pinene and β-myrcene, α-terpinene and cis-ocimene, and between limonene, p-cymene, and trans-ocimene. Even though these monoterpenes are structural isomers (with the exception of monoterpenoid p-cymene), they all produce unique absorbance spectra (Figure 2). Because VUV absorbance is directly proportional to the concentration of analyte passing through the flow cell, these unique absorbance spectra can be used to deconvolve co-eluting peaks accurately (Figure 3).

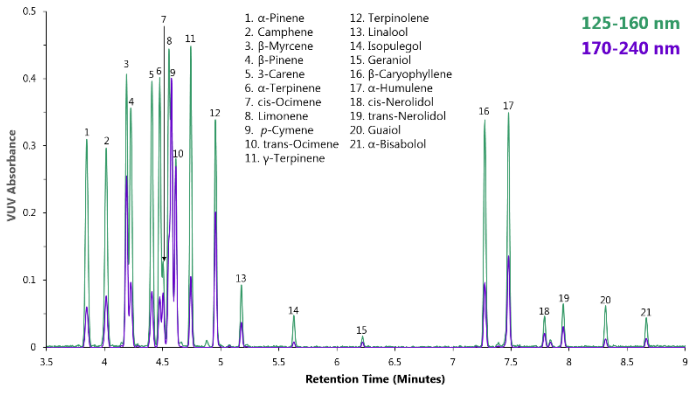

Figure 1: Chromatogram of a terpenes standard mix (two spectral filters shown for visualization purposes). The last terpene compound elutes before 9 min.

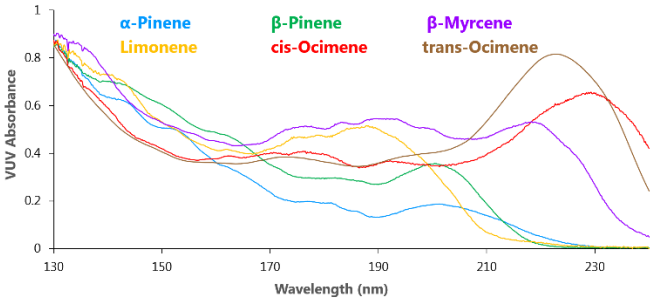

Figure 2: Absorbance spectra of 6 monoterpene isomers. Though some similarities exist between closely related compounds (e.g., α- and β-pinene, cis- and trans-ocimene), each spectrum is still easily distinguished.

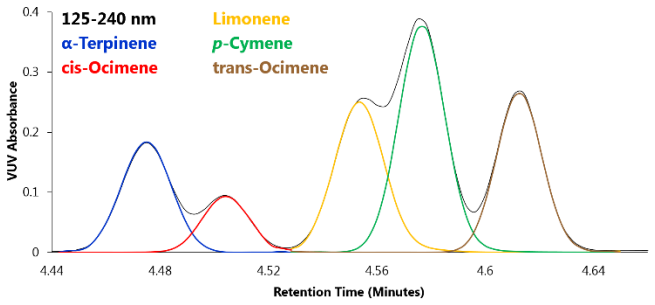

Figure 3: The deconvolution of co-eluting terpenes during the fast GC-VUV run. Unique VUV spectra allow the unambiguous resolution of overlapping peaks α-terpinene and cis-ocimene as well as limonene, p-cymene, and trans-ocimene. VUV absorbance is directly proportional to the concentration of analyte passing through the flow cell, enabling the accurate quantitation of analytes performed by VUV software during post-run analysis.

Figure 4 provides an example of how deconvolution is performed for limonene and p-cymene using their spectral responses. The individual spectra of the terpenes are shown in Panel A along with the summed absorbance of the selected retention time window (blue region in Panel B) and the spectral fit with VUV library reference spectra. The R^2 >0.999 fit result confirms the correct compound identification by the VUV library. This fitting procedure performed by VUV software enables the accurate deconvolution of co-eluting terpenes analyzed by the fast GC-VUV method.

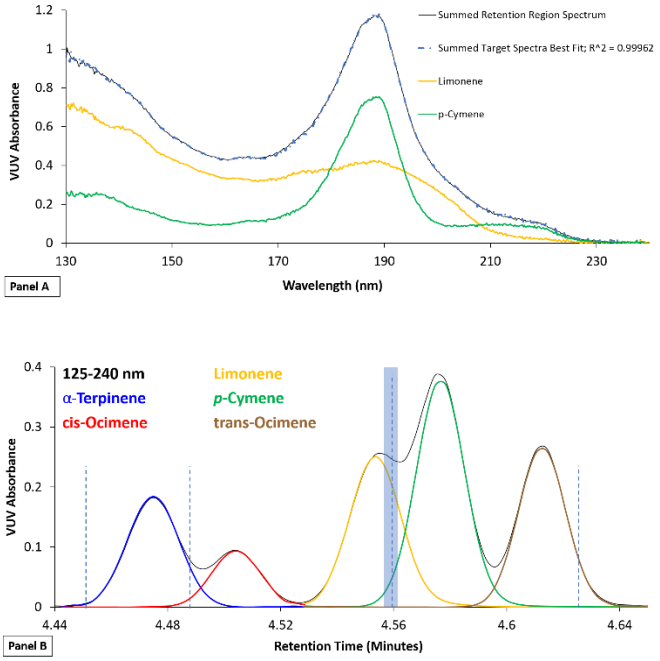

Figure 4: Panel A shows the individual spectra of limonene and p-Cymene along with the summed absorbance of the selected retention time (blue region in Panel B) and the fit with VUV library reference spectra. The deconvolution of five co-eluting terpenes is featured in Panel B.

➢ GC-VUV Analysis of Essential Oils

Essential oils are concentrated hydrophobic solutions of aroma compounds, or the "essence" of a plant's fragrance. Typical methods of terpene extraction include steam distillation, solvent extraction, and cold pressing of the plant matter. Some terpene compounds are artificially synthesized.[5] Six essential oils were analyzed by GC–VUV and static headspace: eucalyptus, lavender, neroli, peppermint, sweet orange, and tea tree. A 5 to 50-µL aliquot of each oil was spiked into 2 mL of water in a 20-mL headspace vial and run under the GC–VUV and static headspace method conditions. The essential oils with simple odor profiles, such as sweet orange and peppermint, had very few major peaks of interest as shown in Figure 5. Oils with more complex profiles had several major peaks, as observed in Figures 6 and 7 with the proportion of cis- and trans-ocimene, linalyl acetate, and linalool in lavender as well as α-pinene with α- and γ-terpinene in tea tree.

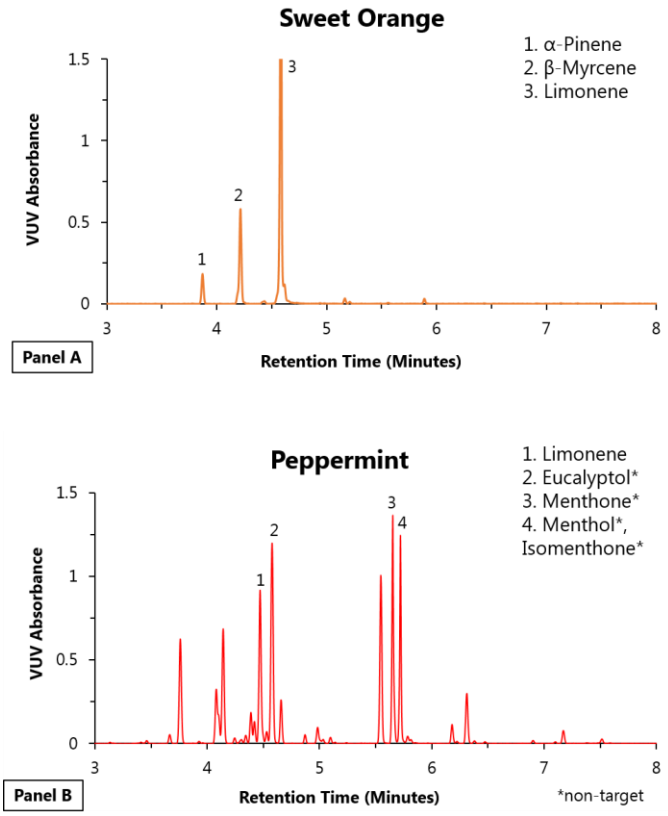

Figure 5: GC-VUV chromatogram of essential oils sweet orange and peppermint. Panel A shows that limonene is the predominant terpene in sweet orange. In Panel B it can be observed that menthol and its derivatives, along with eucalyptol, are in the greatest proportion within the peppermint essential oil. The asterisk indicates untargeted terpenes that were identified during the analysis.

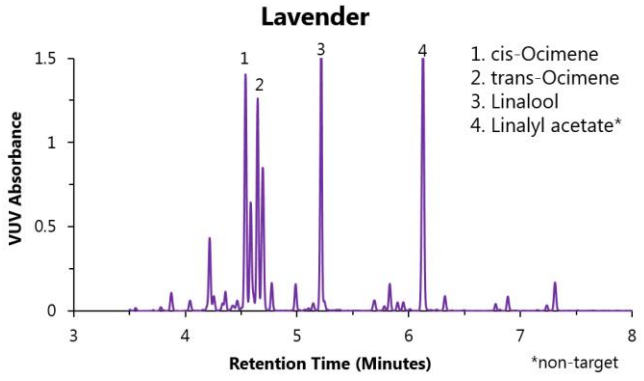

Figure 6: GC-VUV chromatogram of lavender essential oil. The terpenes with the highest relative concentrations were cis- and trans-ocimene, linalyl acetate, and linalool. The asterisk indicates untargeted terpenes that were identified during the analysis.

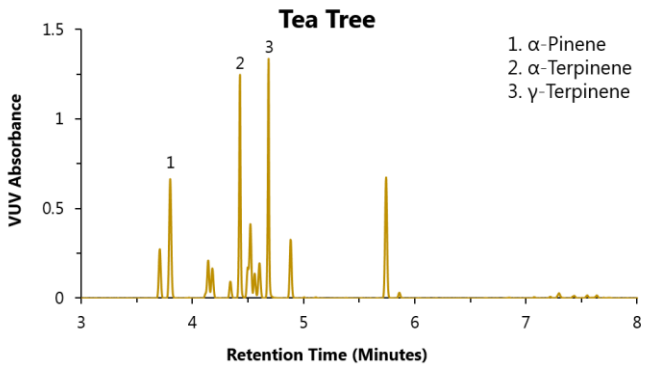

Figure 7: GC-VUV chromatogram of tea tree essential oil. The terpenes found in greatest proportion were α-pinene along with α- and γ-terpinene.

An unexpected result observed in this analysis was that nerol and trans-nerolidol were not detected in the neroli oil. One possible explanation for their absence was the inability to partition them into the headspace due to their solubility with the water matrix. This hypothesis would appear to be supported by the compositional analysis that used liquid injections which is described below. Table 1 displays the quantitative analysis (in µg per gram oil) of the essential oils analyzed. The major chromatogram peak concentrations are highlighted, and their relative proportions correlate to the terpenes most influencing the essential oil properties.

	Eucalyptus	Lavender	Neroli	Peppermint	Sweet Orange	Tea Tree
α-Pinene	15400	346	96.7	2150	3530	14300
Camphene		210	9.29	53.4		
β-Myrcene	6430	996	212	768	7660	3690
β-Pinene	3630	285	976	2040	1010	2800
3-Carene	3100		21.1		331	
α-Terpinene	1340	642	23.3	624		31200
cis-Ocimene	246	13200	286	1430		123
Limonene	18600	1750	865	2470	246000	3960
p-Cymene	2630	428	6.99	178	5522	8950
trans-Ocimene	6070	4360	468	289	967	2920
γ-Terpinene	2280	255	10.5	780		58500
Terpinolene	584	436	22.0	217		8900
Linalool	2340	80000	11900	1060	2850	
Geraniol			1340			
β-Caryophyllene	662	1010		413		880
Nerol			0			
trans-Nerolidol			0			

Table 1: Quantitative GC-VUV and static headspace analysis of terpenes in essential oils (µg per gram oil). The major chromatogram peak concentrations are highlighted, and their relative proportions correlate to the terpenes most influencing the essential oil properties.

- Automated Compositional Analysis of Essential Oils using VUV Analyze™

An alternative approach to quantitating terpenes was developed using the compositional analysis capabilities of VUV Analyze™ software. The automated data analysis package implements equations and fit procedures that utilize a time interval deconvolution (TID) algorithm to divide a chromatogram into equal, small time intervals (typically <0.05 min). For each time interval the measured spectrum is compared against reference spectra in the designated VUV library, and the best analyte(s) fit is determined. The software quickly measures the total response per analyte for a given chromatogram during its data processing procedure. The results are given in relative mass or volume percentage and can be converted into absolute concentration.

A standard mixture of terpenes was analyzed by liquid 1-µL injections. Figure 8 displays two different time ranges of the resulting GC-VUV run as viewed in the VUV Analyze™ chromatogram window. The vertical bar shading represents the relative proportion of each terpene in the sample. The deconvolution of 3-carene, α-terpinene, p-cymene, limonene, and cis-ocimene can be seen in Panel A, and the resolved co-elution of terpinolene and linalool can be observed in Panel B. The quantitative analysis of the standard mixture is shown in Table 2.

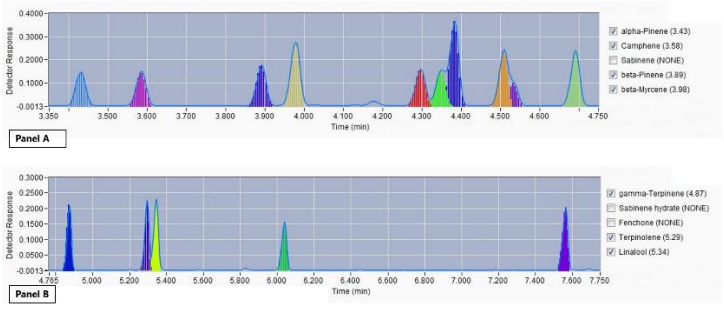

Figure 8: GC-VUV chromatogram of a standard terpene mixture. The deconvolution of 3-carene (red), α-terpinene (green), p-cymene (blue), limonene (orange), and cis-ocimene (purple) can be seen in the VUV Analyze™ chromatogram window displayed in Panel A, the resolved co-elution of terpinolene and linalool can be observed in Panel B.

Terpenes Standard Mixture			
Analyte	Mass %	Analyte	Mass %
α-Pinene	5.15	Isopulegol	4.73
Terpinolene	5.03	Linalool	4.72
β-Pinene	5.01	Geraniol	4.67
γ-Terpinene	4.91	cis-Ocimene	4.66
Camphene	4.90	β-Caryophyllene	4.65
cis-Nerolidol	4.83	α-Humulene	4.65
Limonene	4.83	β-Myrcene	4.64
3-Carene	4.83	trans-Nerolidol	4.58
p-Cymene	4.79	α-Bisabolol	4.53
α-Terpinene	4.77	Guaiol	4.37
trans-Ocimene	4.75		

Table 2: Quantitative GC-VUV analysis of standard terpene mixture using VUV Analyze™ compositional analysis. Values are given in relative mass %, but relative volume % and absolute concentration can be determined by applying density and external calibration curve information, respectively.

The same methodology was applied to the characterization of essential oils. Table 3 displays the quantitative analysis of eucalyptus. Table 4 contains the results of neroli oil analysis. Small quantities of nerol and trans-nerolidol were detected using the liquid injection strategy. As mentioned previously, these compounds did not appear to be amenable to headspace partitioning from the water matrix used in the experiment. The relative mass of each compound was still lower than expected when compared to the reported literature values.

Eucalyptus Essential Oil			
Analyte	Mass %	Analyte	Mass %
Eucalyptol	68.46	β-Pinene	0.62
α-Terpineol	12.66	Sabinene	0.59
Limonene	4.71	Geraniol	0.58
4-Terpineol	2.69	Linalool	0.37
α-Pinene	2.49	α-Terpinene	0.31
cis-Ocimene	1.37	Terpinolene	0.17
β-Myrcene	1.28	trans-Citral	0.16
α-Phellandrene	1.05	β-Caryophyllene	0.12
trans-Ocimene	0.85	cis-Citral	0.06
p-Cymene	0.80	Nerol	0.03
γ-Terpinene	0.62		

Table 3: Quantitative GC-VUV analysis of eucalyptus essential oil using VUV Analyze™ compositional data analysis.

Neroli Essential Oil	
Analyte	Mass %
Linalool	51.68
Linalyl acetate	8.34
Limonene	7.46
α-Terpineol	7.09
β-Pinene	6.82
trans-Ocimene	4.27
Geraniol	3.63
Geranyl acetate	2.74
trans-Citral	2.25
Nerol	1.98
cis-Ocimene	1.95
trans-Nerolidol	1.51
β-Caryophyllene	0.28

Table 4: Quantitative GC-VUV analysis of neroli oil using VUV Analyze™ compositional data analysis. Small percentages of nerol and trans-nerolidol were detected as a result of liquid injection, demonstrating the difficulty in partitioning these compounds into headspace in the presence of a water matrix.

Conclusion

A fast GC–VUV and static headspace method was used to identify and quantitate terpenes. The uniqueness of VUV absorbance spectra, along with the fact that the VUV detector is used at ambient pressure and thus not flow rate limited, allows for deliberate chromatographic compression and shorter GC run times. This GC–VUV method analyzed 21 terpenes in less than 9 min. Co-eluting peaks were deconvolved using VUV absorbance spectra, leading to accurate identification and quantitation of terpene compounds. This fast GC–VUV method extends beyond the realm of terpenes. Any application where chromatographic compression is a viable option, or where coeluting isomer identification is a priority, would benefit from this approach.

A GC-VUV liquid injection method and alternative quantitative approach to characterizing terpenes was also introduced. The liquid injection method proved especially helpful in detecting compounds not amenable to headspace partitioning. The unique capability of automating compositional analysis of terpenes in mixtures using VUV Analyze™ software significantly reduces data processing time and the potential for error in interpretation. This approach has already been demonstrated to be effective in analyzing hydrocarbons in gasoline streams, and will undoubtedly revolutionize how terpenes are characterized within the flavor and fragrance, cosmetic, and pharmaceutical industries.

For more detailed information please visit our website at www.vuvanalytics.com, or contact us at info@vuvanalytics.com

Acknowledgements

Alex Hodgson & Jack Cochran, VUV Analytics and Kevin Schug, Department of Chemistry & Biochemistry, University of Texas at Arlington

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www.vuvanalytics.com

Science in a New Light

VUV Analytics, Inc.

Austin, TX

(512) 333-0860

www.vuvanalytics.com

info@vuvanalytics.com

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