Potential Allergens in Aromatherapy Oils by GC/MS Using an Agilent J&W DB-XLB Capillary Column

Application Note

Consumer Products and Flavors and Fragrances

Author

Ken Lynam
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808
USA

Abstract

The European Union (EU) regulates 26 flavor and fragrance allergens. Twenty-four of these allergens are amenable to analysis by GC/MS. Some of these allergens are present at appreciable levels in essential oils used in aromatherapy. This note demonstrates a single quad GC/MS analysis of the GC/MS amenable listed allergens using the unique selectivity of an Agilent J&W DB-XLB capillary GC column. Representative total ion chromatograms of ylang ylang, lavender, and eucalyptus oils are shown. The DB-XLB column easily resolved the 24 analytes of interest, having both excellent peak shapes and high sensitivity.

Agilent Technologies

Introduction

The European Union (EU) regulates 26 flavor and fragrance allergens. GC/MS is an effective analytical technique for 24 of the 26 compounds listed as EU flavor and fragrance allergens. The GC/MS-amenable allergens (Table 1) are sufficiently volatile for this approach. Oak and tree moss extracts (the 2 remaining listed allergens) are less volatile and require LC/MS for effective analysis. The 24 GC/MS-amenable allergens are often components in fragrance, cosmetic and aromatherapy product formulations. Listed allergen limit targets are in place in the EU for rinse-off and leave-on fragrance and cosmetic products in the 10 – 100 ppm range [1]. Identification and detection of the listed allergens at these levels is readily obtainable using a single quad GC/MS approach. Acceptable levels and labeling requirements for aromatherapeutic products is less clear. These products can contain EU-listed allergens at levels more than ten times the acceptable level of 10 ppm for leave-on cosmetic products such as ointments and creams. Leave-on and rinse-off limits established for fragrance and cosmetic products serve as useful guides for analysis of aromatherapy products.

Professionals in the aromatherapy and massage industries strongly discourage the use of neat essential oils. These oils are strong concentrates that may contain irritants, sensitizers, and even potential carcinogens in addition to allergens [2]. Fortunately, these concentrated oils are typically mixed with carrier oils such as vegetable or mineral oil prior to use. Recipes for dilution often call for 1% to 5% dropwise preparation of the neat essential oils dispersed in the carrier oil. This practice only partially mitigates the potential harm from exposure to the chemicals present in the neat oils. It is imperative that both the practitioner providing the therapy and the patient having these products applied to their skin are aware of the potential risks present in order to use these products safely and effectively.

Continuous improvements in column manufacturing technology and GC/MS instrumentation have made the evaluation of aromatherapy oils a simpler and more reliable process. Detection and identification of the potential allergy-inducing components in these oils down to the single part per million (ppm) range is readily achievable. The quality of capillary GC/MS columns in terms of their bleed and surface activity profiles dramatically enhance the performance of this type of analysis with better analyte resolution and sharper peak shapes. Design improvement in GC/MS systems work with the improved columns to enhance GC/MS analyses in the low ppm range and become standard practice. Routine analysis of these oils for the 24 GC/MS-amenable allergens is now both a straightforward and robust process, if the peaks of interest can be resolved from matrix components. This application note highlights the separating power and chromatographic performance available today using Agilent J&W DB-XLB columns coupled with an Agilent 5975C series GC/MSD, capable of simultaneous SIM and Scan mode spectrometry.

Previous studies of this allergen set by GC/MS focused primarily on fragrance and cosmetic products. Several approaches have been described. The first one uses a deconvolution reporting software (DRS) approach on an Agilent J&W HP-5ms column. The second uses a two-dimensional column approach with a low thermal mass (LTM) device and Deans switch heart cutting to an Agilent J&W DB-17ms column. Finally, the third uses a single DB-17ms column single quad GC/MS approach to evaluate allergens in snack foods [3–5]. The focus of this application is a single column GC/MS separation of the allergens using the selectivity offered by the DB-XLB stationary phase. The selectivity of the midpolarity DB-XLB column is a useful tool in helping to resolve the allergens away from potential matrix interference. The sample matrices here are the essential oils used in the aromatherapy and massage industries.

Table 1. GC/MS-amenable EU Flavor and Fragrance Allergens

1	Limonene	7	Citral	13	Isoeugenol	19	Farnesol
2	Benzyl alcohol	8	Hydroxy cittronellal	14	a-isomeyhyl ionone	20	Amyl cinnamyl alcohol
3	Linalool	9	Anisyl alcohol	15	Coumarin	21	Hexyl cinnamaldehyde
4	Folione	10	Cinnamaldehyde	16	Lilial	22	Benzyl benzoate
5	Citronellol	11	Cinnaminic alcohol	17	Amyl-cinnamaldehyde	23	Benzyl salicilate
6	Geraniol	12	Eugenol	18	Lyral	24	Benzyl cinnamate

Experimental

This analysis was done with an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.25 µm column (Agilent p/n 122-1232) on an Agilent 7890 GC System, together with an Agilent 5975C series GC/MSD. Details of the chromatographic conditions are shown in Table 2. Details of the flow path supplies used are listed in Table 3.

Table 2. Chromatographic Conditions for EU Allergen Analysis

GC/MS:	Agilent 7890A GC System, Agilent 5975C series GC/MSD, Triple Axis Detector
Sampler:	Agilent 7683B automatic liquid sampler, 5.0 µL syringe (Agilent p/n 5183-4729)
Injection:	1.0 µL
Carrier:	Helium, fixed pressure 11.06 psi
Inlet:	50:1 split ratio 250 °C, total flow 70.73 mL/min, 3 mL/min septum purge, gas saver on, 50 mL/min after 2 minutes
Inlet Liner:	MS certified liner (Agilent p/n 5188-6568)
Column :	Agilent J&W DB-XLB 30 m x 0.25 mm x 0.25 µm (Agilent p/n 122-1232)
Oven:	50 °C (1.0 min) to 100 °C (8 °C/min); 2 °C/min to 110 °C (2 min), 5 °C/min to 185, 30 °C/min to 280 °C (3 min)
MSD:	Transfer line 310 °C, source 350 °C, quadrupole 180 °C

Table 3. Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial Caps:	Screw caps (Agilent p/n 5182-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5183-4729)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet Seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
Inlet liners:	MS certified liner (Agilent p/n 5188-6568)
Ferrules:	0.4 mm id short; 85/15 Vespel/graphite (Agilent p/n 5181-3323)
20x magnifier:	20x Magnifier loop (Agilent p/n 430-1020)

Sample Preparation

Twenty-four individual 1000 ng/µL EU GC/MS flavor and fragrance standard solutions, obtained from AccuStandard, New Haven, CT were combined in equal portion with a laboratory prepared 1000 ng/µL internal standard solution to form a 40 ng/µL combined allergen standard solution. The internal standard 1,4 dibromobenzene was purchased from Sigma Aldrich, St. Louis, MO and prepared at a concentration of 1000 ng/µL in acetonitrile. The 40 ng/µL combined allergen solution was diluted 1:4 in acetonitrile to make a standard at a concentration of 10 ng/µL. Standards were prepared fresh, stored at 5 °C and used within seven days of preparation.

Ylang ylang, lavender and eucolyptus essential oil samples were obtained from a local retail store. All of the essential oils were labeled aromatherapeutic GC/IR verified grade and designated 100% pure and natural. The essential oil samples were diluted 1:20 in acetone and prepared fresh the day of the analysis.

Results and Discussion

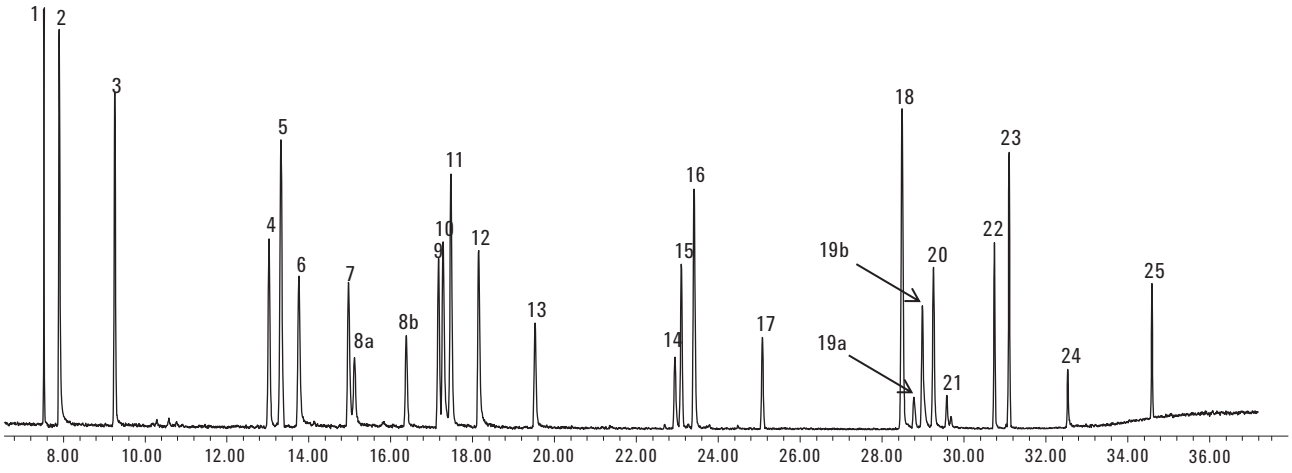
Most of the EU listed allergens fall into two chemical categories; aldehydes and alcohols. Both of these chemical classes of compounds are comprised of active analytes that can often lead to poor chromatographic performance. Using the DB-XLB column, excellent resolution and peak shape was achieved for these analytes.

Figure 1 shows the separation of 24 GC/MS-amenable allergens on a DB-XLB 30 m × 0.25 mm × 0.25 µm capillary GC column at a concentration of 40 ppm. Factoring in the 1-µL volume and a split ratio of 50:1 on-column loading of these analytes is 0.8 ng/component. This figure clearly shows that all 24 GC/MS-amenable allergens are easily detectable and identifiable at this level.

In this standard set citral, lyral, and farnesol have more than one isomer each and appear as more than one peak. Peak 19b is a combination of lyral 2 and farensol 1. Fortunately, the peaks for lyral 1 and farnesol 2 resolve nicely on the DB-XLB column enabling confirmation of either of these two analytes in the presence of each other over the 10–100 ppm range studied.

The dibromobenzene internal standard is included at the same concentration in both the standard and sample chromatograms to provide a fixed frame of reference in each of the figures. The Y scale in Figure 1 is magnified 25 times above the Y scale in the sample figures to highlight the sensitivity and peak shapes obtained with standard injection. The naturally occurring essential oil samples contain a wide variety of more concentrated components, plus the dibromobenzene internal standard at the same concentration as in the standard.

0.8 ng/component loading of 24 EU Allergens on an Agilent J&W DB-XLB Column

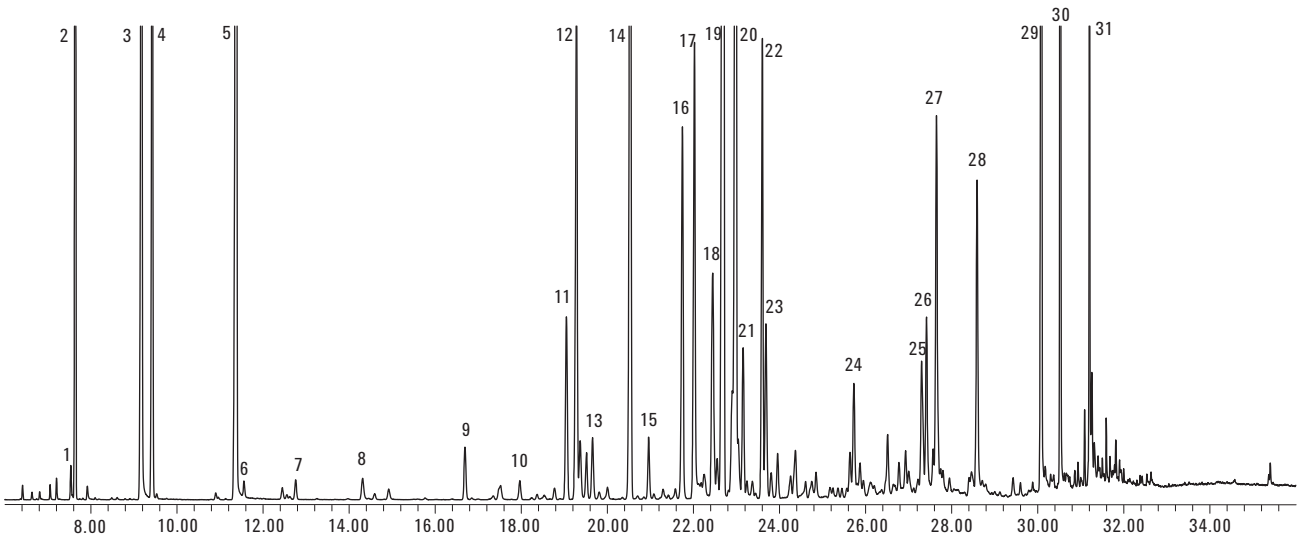


EU Flavor and Fragrance Allergens

1	Limonene	8a	Citral (neral)	14	Isoeugenol	19b	Farnesol 1
2	Benzyl alcohol	8b	Citral (geranial)	15	α-Isomethyl ionone	20	Amyl cinnamyl alcohol
3	Linalool	9	Hydroxy citronellal	16	Coumarin	21	Farnesol 2
4	Folione	10	Anisyl alcohol	17	Lilial	22	Hexyl cinnamaldehyde
5	Dibromobenzene (ISTD)	11	Cinnamaldehyde	18	Amyl cinnamaldehyde	23	Benzyl benzoate
6	Citronellol	12	Cinnaminic alcohol	19	Lyral 1	24	Benzyl salicilate
7	Geraniol	13	Eugenol	19b	Lyral 2	25	Benzyl cinnamate

Figure 1. Total ion chromatogram of a 1-µL injection of 40 ppm standard solution containing 24 EU allergens on an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.25 µm column (p/n 122-1232). Y scale in this figure ranges from 0 to 2 × 10^3 counts, peak number 5 is the internal standard. GC conditions are in Table 2.

Figure 2 shows a total ion chromatogram of an ylang ylang essential oil sample diluted 1:20 in acetonitrile. The 1:20 dilution is representative of a typical 3% to 5% dropwise preparation for an essential oil in a mineral oil carrier. It serves as an excellent reference point for evaluating the allergen risk potential of an essential oil. The EU allergens detected and identified in this sample are in bold type below the figure. In this sample linalool, cinnamyl acetate, benzyl benzoate, and benzyl salicilate were all present. These potential allergens were present at levels more than 100 times the low level or 10 ppm investigated in this application.



Ylang Ylang Oil Major Components include 4 EU Allergens

1	Eucolyptol	9	p-Propyl anisol	17	***Cinnamyl acetate***	25	δ-Muurolene
2	p-Methyl anisol	10	α-Cubebene	18	t-Muurolene	26	t-Cardinol
3	***Linalool***	11	Copaene	19	Germacrene D	27	t-Muurolol
4	Methyl benzoate	12	β-Myrcene	20	α-Farnesene	28	β-Farnesene
5	Benzyl acetate	13	β-Elemene	21	α-Muurolene	29	***Benzyl benzoate***
6	Ethyl benzoate	14	Caryophyllene	22	δ-Cadinene	30	Caryophyllene
7	Dibromobenzene (ISTD)	15	β-Cubebene	23	γ-Cadinene	31	***Benzyl salicylate***
8	Cic-gernaniol	16	α-Caryophyllene	24	Carphyllene oxide		

Figure 2. Total ion chromatogram of ylang ylang oil diluted 1:20 in acetonitrile on an Agilent J&W DB-XLB ms 30 m x 0.25 mm × 0.25 µm column (p/n 122-1232). Y scale in this figure ranges from 0 to 5 x 10^6 counts, peak number 7 is the internal standard. GC conditions are in Table 2.

Figure 3 displays a total ion chromatogram of a lavender essential oil sample diluted 1:20 in acetonitrile. Linalool was present in this sample. Linalool is a main component in lavender oil, in this case, 1000 times the level of the 10 ppm standard.

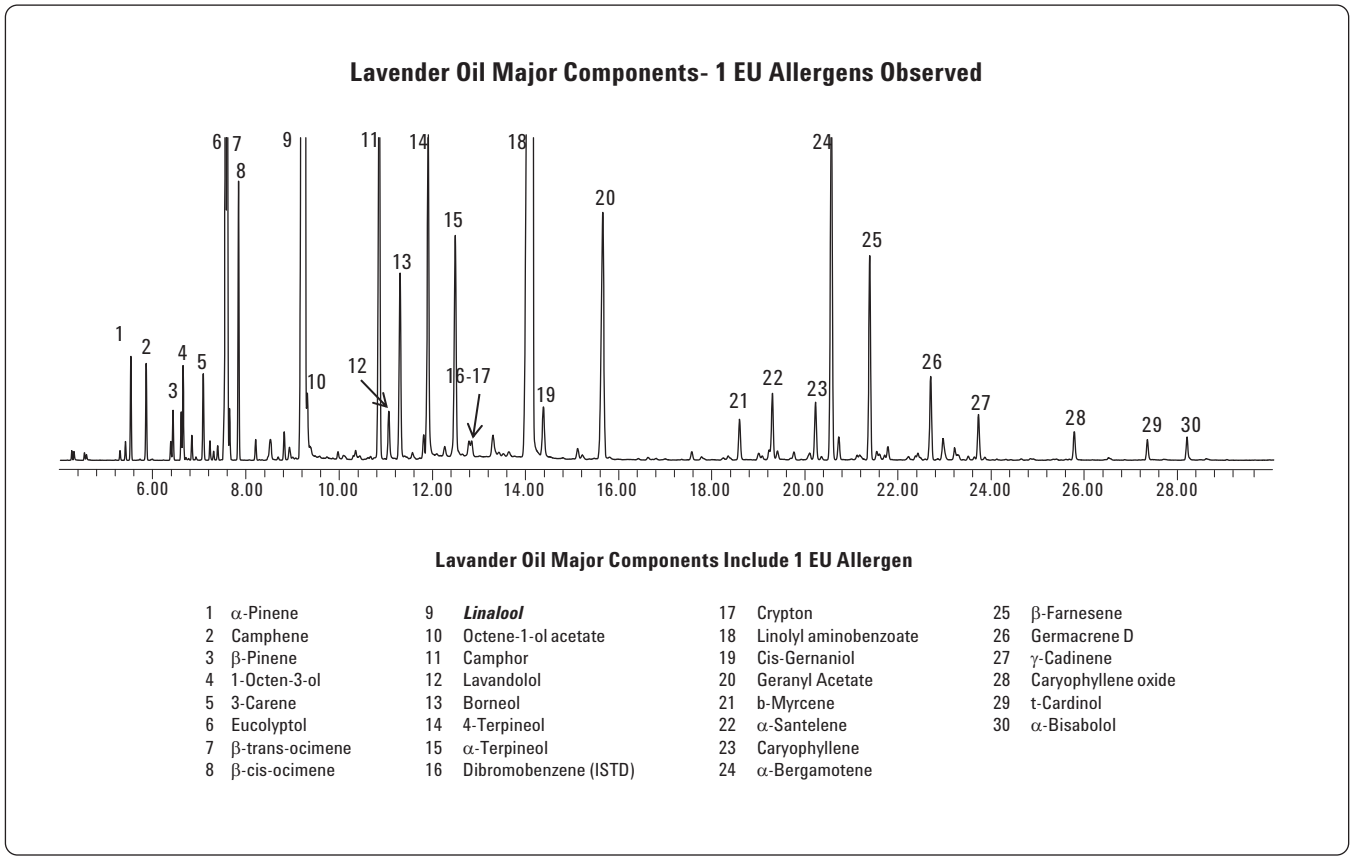


Figure 3. Total ion chromatogram of lavender oil diluted 1:20 in acetonitrile on an Agilent J&W DB-XLB ms 30 m × 0.25 mm × 0.25 µm column (p/n 122-1232). Y scale in this figure ranges from 0 to 5 x 10^6 counts, peak number 16 is the internal standard. GC conditions are in Table 2.

Figure 4 is a TIC of a eucalyptus essential oil sample diluted 1:20 in acetonitrile. In this sample, a single EU allergen was detected and identified. Linalool was present in this sample at a level approximately 5 times the 10-ppm standard. Eucalyptus oil was the simplest and most volatile of the essential oils investigated.

Conclusions

At least one EU-regulated allergen was identified in each of the essential oil samples investigated in excess of the 10-ppm limit set by the EU for leave-on cosmetic products. In ylang ylang and lavender oils, several of these allergens were present at more than 100 times the target limit for the compounds even for samples diluted 1 to 20. The 1 to 20 dilution chosen is representative of a typical 3% to 5% dropwise preparation done by massage and aromatherapists. It is imperative that professionals in the aromatherapy and massage industries are aware of the potential risks for these allergens to produce allergic responses, even in diluted form. Close monitoring by GC/MS analyses, appropriate labeling, and careful dilution of these products are required for safe use.

This application note successfully demonstrates the utility of a single quad GC/MS approach using an Agilent J&W DB-XLB column for analysis of the EU flavor and fragrance allergens in aromatherapy oils. Using the GC/MS conditions described, 24 of the EU-listed allergens amenable to GC/MS analysis are detectable and identifiable. Identification at the 10-ppm level, which is the leave-on limit set for fragrance and cosmetic products in the EU directive, was easily achievable.

Analyte peaks on the DB-XLB column were well resolved and sharp considering the active nature of the alcohol and aldehyde components in this sample set. The resolution observed on this column for the allergen peaks shows that reliable and robust analysis of potential allergen components in essential oils is both achievable and straightforward.

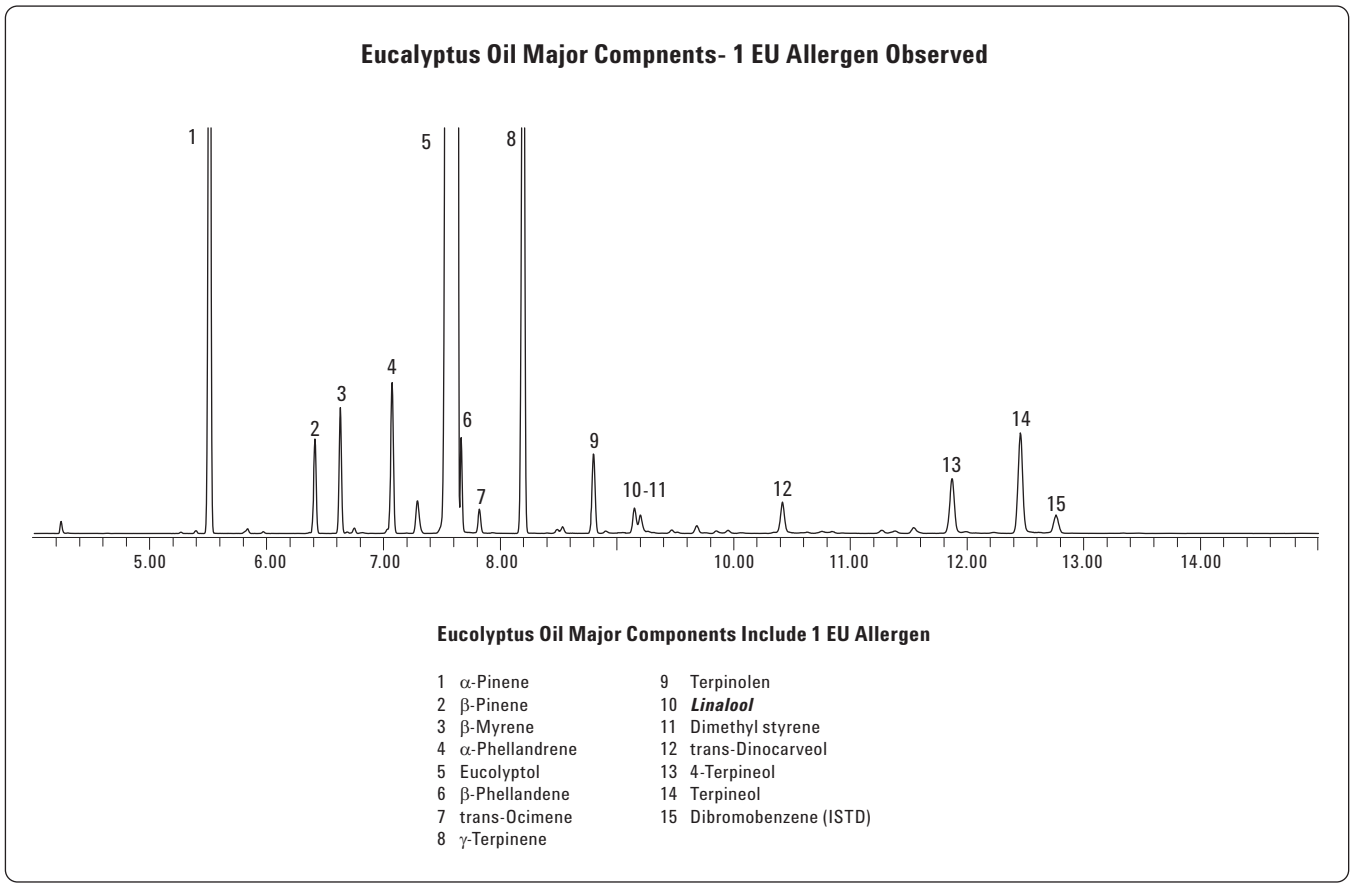


Figure 4. Total ion chromatogram of eucalyptus oil diluted 1:20 in acetonitrile on an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.25 µm column (p/n 122-1232). Y scale in this figure ranges from 0 to 5 x 10^6 counts, peak number 15 is the internal standard. GC conditions are in Table 2.

Reference

1. EU Directive 2003/15/EC, Official Journal of the European Union, 6 66.26, 11.3.2003
2. Sue Clark, Editor "Essential Chemistry for Aromatherapy" 2nd Edition, ISBN-13: 978-0-443-10403-9, Feb. 2009, Elsevier Press, Chapter 8, pages 231-264
3. W. Luan, C. Sandy, and M. Szelewski, "Determination of Allergens in Fragrance Products Using Agilent Deconvolution Reporting Software," Agilent Technologies publication 5989-8723EN, June 2008
4. F. David and M.S. Klee, "Analysis of Suspected Flavor and Fragrance Allergens in Perfumes Using Two-Dimensional GC with Independent Column Temperature Control Using an LTM Oven Module," Agilent Technologies publication 5990-3576EN, February 2009
5. Doris Smith and Ken Lynam, "GC/MS Identification of Flavor and Fragrance Allergens in Some Common Snack Foods Using an Agilent J&W DB-17ms Capillary GC Column," Agilent Technologies publication 5990-4784EN, October 2009

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
February 18, 2014
5990-5293EN

Agilent Technologies