GC/MS Identification of Flavor and Fragrance Allergens in Some Common Snack Foods Using an Agilent J&W DB-17ms Capillary GC Column

Application Note

Food

Authors

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Abstract

The European Union (EU) regulates 26 flavor and fragrance allergens. Some of these allergens are flavoring additives in common snack food such as candy and gum. Cinnamon related allergens are of particular interest, encompassing five of the 26 EU listed allergens. Cinnamon flavored gum and candy MTBE extracts show appreciable levels of these listed allergens. All 24 of the GC/MS amenable allergens are resolved and identified on an Agilent J&W DB-17ms column.

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Introduction

The European Union (EU) regulates 26 flavor and fragrance allergens (1). Some of these allergens are flavoring additives in common snack food such as candy and gum. Cinnamon related allergens are of particular interest, encompassing five of the 26 EU listed allergens. Cinnamon flavoring can approach percent levels in some of these intensely flavored food products. These concentrations are well beyond acceptable levels of rinse off or leave limits for topical products such as shampoos, creams and perfumes. Acceptable levels of the flavoring agents are still under debate. When used as a flavoring, cinnamon is typically included under the broad label of natural or artificial flavor with an upper limit percentage descriptor.

The target limit of listed allergens for rinse off products is 100 ppm. The leave on limit is 10 ppm in fragrance and cosmetic products. In this application, these limits were reference points for candy and gum products where ingestion is the chief route of exposure rather than inhalation or adsorption routes typical for fragrance and cosmetic products. Detection at these levels is readily obtainable by single quad GC/MS for the purposes of identification and semiquantitative evaluation. GC/MS is an effective technique for 24 of the 26 compounds listed as EU flavor and fragrance allergens. Oak and tree moss extracts are also listed allergens but are not suitable for GC/MS analysis.

Previous studies of this allergen set by GC/MS focused primarily on fragrance and cosmetic products. Several approaches have been described, one using a deconvolution reporting software (DRS) approach on a HP-5ms column and one using a two-dimensional column approach employing a low thermal mass (LTM) device with Deans switch heart cutting [2,3]. The focus of this application is a single column GC/MS approach to separate the allergens in flavored candy and gum products using the selectivity offered by a DB-17ms. The selectivity of the midpolarity DB17ms column is a useful tool in helping to resolve the allergens away from potential matrix interference.

Experimental

This analysis was done on an Agilent J&W DB-17ms 30 m × 0.25 mm, 0.25 µm column on an Agilent 7890/5975C GC/MS system. Details of the chromatographic conditions are shown in Table 1. Details of the flow path supplies necessary for the analysis are listed in Table 2.

Table 1. Chromatographic Conditions for EU Allergen Analysis

GC/MS:	Agilent 7890A/5975C Triple Axis Detector
Sampler:	Agilent 7683B, 5.0 µL syringe (Agilent p/n 5183-4729)
Injection:	1.0 µL
Carrier:	Helium fixed pressure 13.19 psi
Inlet:	50:1 split ratio 250 °C, total flow 60.351 mL/min, 3 mL/min septum purge, gas saver on, 50 mL/min after 1 minute
Inlet Liner:	MS certified liner (Agilent p/n 5188-6568)
Column :	Agilent J&W DB-17ms 30 m × 0.25 mm, 0.25 µm (Agilent p/n 122-4712)
Oven:	150 °C (0.1 min) to 195 °C (7 °C/min); 15 °C/min to 280 °C, 2 min hold
MSD:	Transfer line 280 °C, source 300 °C, quadrupole 180 °C

Table 2 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:	10 µL (Agilent p/n 5183-4729)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet Seal:	Gold plated inlet seal (Agilent part p/n 5188-5367)
Inlet liners:	MS certified liner (Agilent part 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

Individual 1000 ng/µL EU flavor and fragrance standard solutions from AccuStandard were diluted 1 to 10 in three groups for single point calibration at 100 ng/µL. It was necessary to separate the standards into three groups to achieve a 1:10 dilution. Nine aldehydes and ketones composed the first group, nine alcohols the second and the remaining alcohols and neutral molecules were diluted in the third group. The internal standard 1,4 di-bromo benzene was purchased from Sigma Aldrich, prepared at 1000 ng/µL and diluted 1:10 along with each group of calibration standards. A grand mix of 24 standards plus the internal standard was prepared at a concentration of 40 ng/µL; this mix was diluted 1:5 to form a working level standard at 8 ng/µL.

Samples were ground in a stainless steel coffee grinder to obtain a uniform powder consistency. One-gram cinnamon gum and 10-gram candy samples were weighed to the nearest 0.1 mg into 50-mL centrifuge tubes. A 10-mL amount of HPLC water was added by a class A volumetric pipette, and the tube was capped and vortexed for 30 s to wet the samples with water. A 10-mL amount of methyl tert-butyl methyl ether (MTBE, JT Baker high purity grade) was added by a class A volumetric pipette. The samples were extracted for 30 min on an Eberbach reciprocating shaker set on high speed. Samples were spun down 4000 times to separate the MTBE and aqueous layers. Samples were taken from the top MTBE layer for analysis.

Repeatability of the extraction procedure was investigated using five replicate sample preparations of a cinnamon gum sample. The large amount of cinnamaldehyde present in this sample required a 30:1 dilution to bring the response into range of the standard. A semiquantitative analysis of the amount of cinnamaldehyde on a weight to weight basis in the sample was determined in the dilute solutions.

Results and Discussion

The weight percent result for cinnamaldehyde in cinnamon gum was 0.22 ± 0.01 % for duplicate injections of five replicate weighting with a relative standard deviation of 4.3 %. This result is encouraging and indicates a high level of repeatability for the extraction procedure. Extraction efficiency and accuracy evaluations of the extraction procedure were beyond the scope of this study. Given the encouraging result found in the repeatability evaluation a more thorough investigation of the extraction in terms of efficiency and accuracy is a logical next step.

Figure 1 shows the separation of 24 GC/MS-amenable allergens on a DB-17ms, 30 m × 0.25 mm, 0.25 µm capillary GC column at a concentration of 8 ng/µL. This figure clearly shows that all 24 GC/MS-amenable allergens are detectable and identifiable 20 % below the leave on limit using a DB-17ms column and a 50:1 split injection. Reduction of the split ratio could achieve lower limits of detection at the expense of a higher matrix background effect. Backflusing using a capillary flow device (CFT) of late-eluting components would be one way of counteracting deleterious matrix effects and increasing sample throughput.

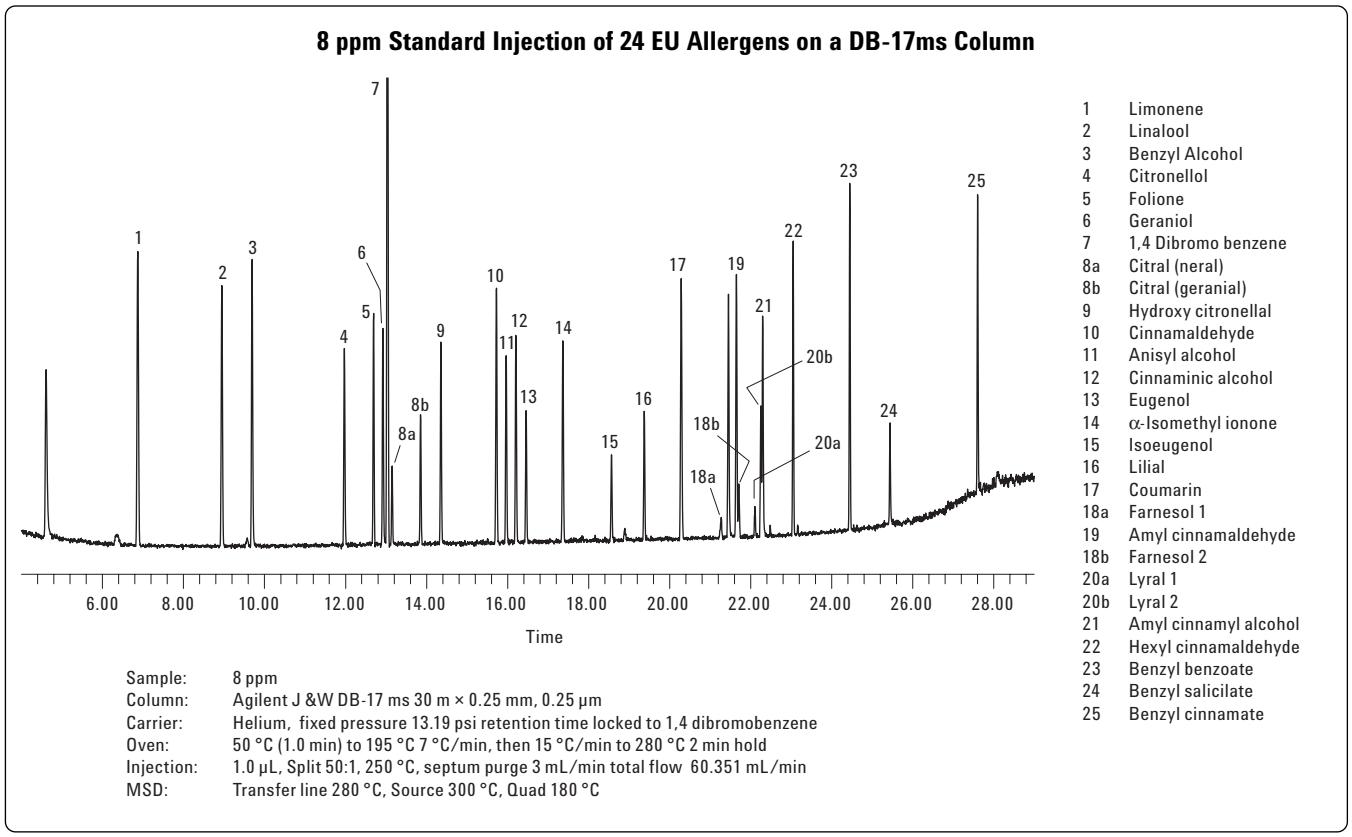

Figure 1. The 8 ppm standard injection of 24 EU allergens on an Agilent J&W DB-17ms, 30 m × 0.25 mm, 0.25 µm column (p/n 122-4712). GC conditions are in Table 1.

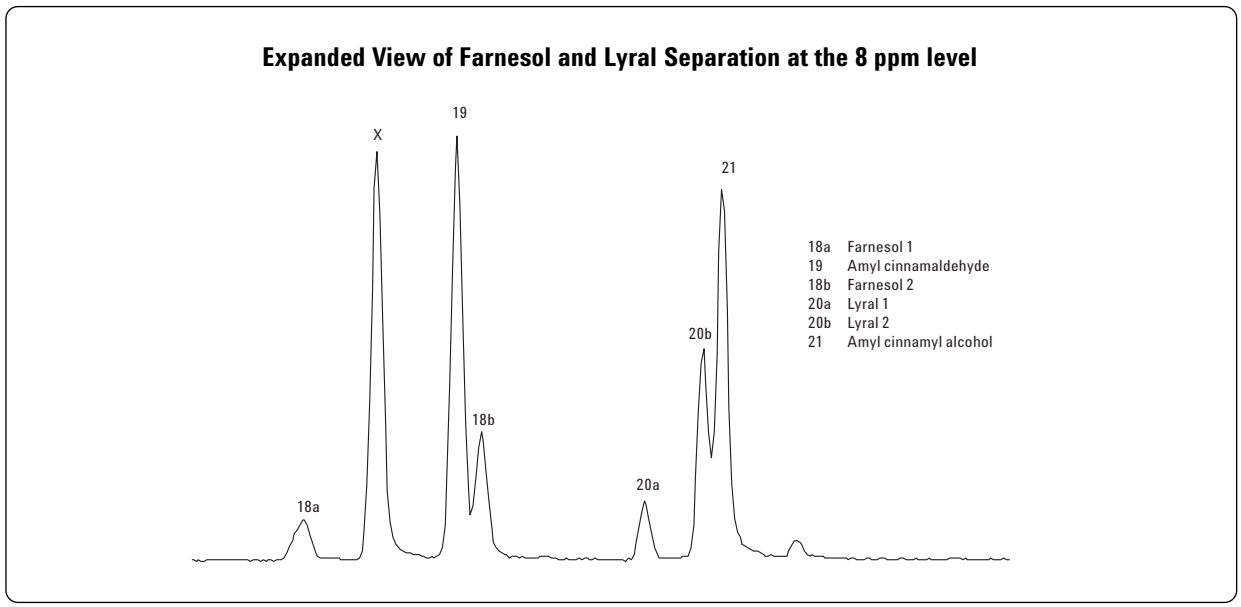

Figure 2. Expanded view of farnesol and lyral separation at the 8 ppm level on a Agilent J&W DB-17ms, 30 m × 0.25 mm, 0.25 µm column (p/n 122-4712). GC conditions used are in Table 1. The x label peak is a contaminant present in the 24-allergen mix and not an analyte of interest.

Citral, farnesol and lyral are all isomeric mixtures and under these conditions two peaks were observed for each of these analytes. Figure 2 is an expanded view of the farnesol and lyral isomers from amyl cinnamaldehyde and amyl cinnamyl alcohol in the 8 ppm, 24-mix standard. One isomer for both farensol and lyral analytes is baseline resolved. The second farnesol peak (18a) is partially resolved from the tail of the amyl cinnamaldehyde peak (19). The second lyral peak (20b) is partially resolved from the front of the amyl-cinnamyl alcohol peak (21). Given the unique elution patterns and mass spectral properties of these analytes, effective identification is achievable at these levels.

Figure 3 is an example chromatogram of one of the neat cinnamon gum extracts. Benzyl alcohol, cinnamaldehyde, cinnaminic alcohol, and eugenol are present in this sample at detectable and identifiable levels. Cinnamaldehyde is present at a level nearly 30 times the 100-ppm limit. It was necessary to dilute this extract 30:1 to bring the signal for cinnamaldehyde in range for quantification.

Figure 4 is an example chromatogram of a neat cinnamon candy extract in MTBE. Limonene, benzyl alcohol, cinnamaldehyde, eugenol, and benzyl benzoate are present in this sample at detectable and identifiable levels. Cinnamaldehyde is present at a level more than 50 times the 100-ppm standard in this sample.

Conclusions

A simple MTBE extraction procedure produced a high degree of repeatability for allergen analysis in a cinnamon flavored gum sample. The extraction procedure shows promise for allergen analyses in difficult matrices such as chewing gum and candy. Perhaps a solid phase extraction approach using MTBE as a solvent would be a useful next step in more fully characterizing the preparation of these samples.

This application successfully demonstrates the utility of a single quad GC/MS approach using an Agilent J&W DB-17ms column for analysis of the EU flavor and fragrance allergens in candy and gum samples. Using the GC/MS conditions described, 24 of the EU listed allergens amenable to GC/MS analysis are detectable and identifiable. Identification at 8 ppm, 20 % below the leave on limit set for fragrance and cosmetic products in the EU directive, was easily achievable.

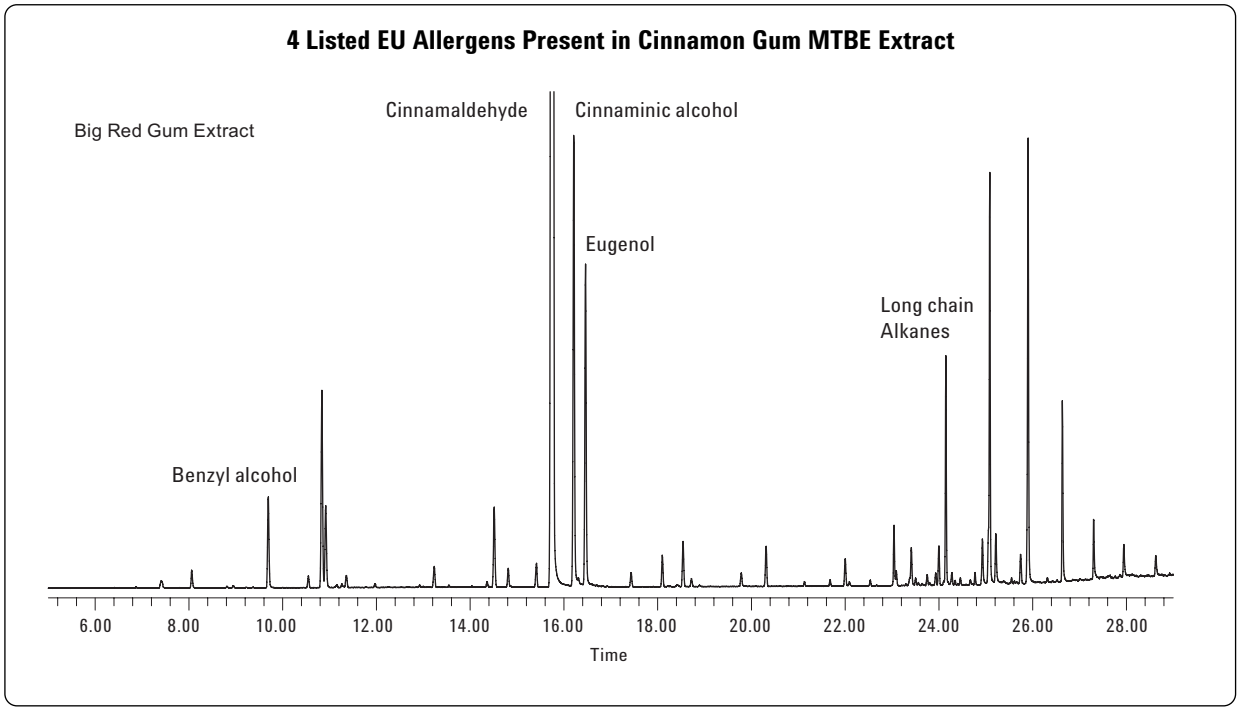

Figure 3. Neat MTBE cinnamon gum extract injection on a Agilent J&W DB-17ms, 30 m × 0.25 mm, 0.25 µm column (p/n 122-4712). GC conditions used are in Table 1.

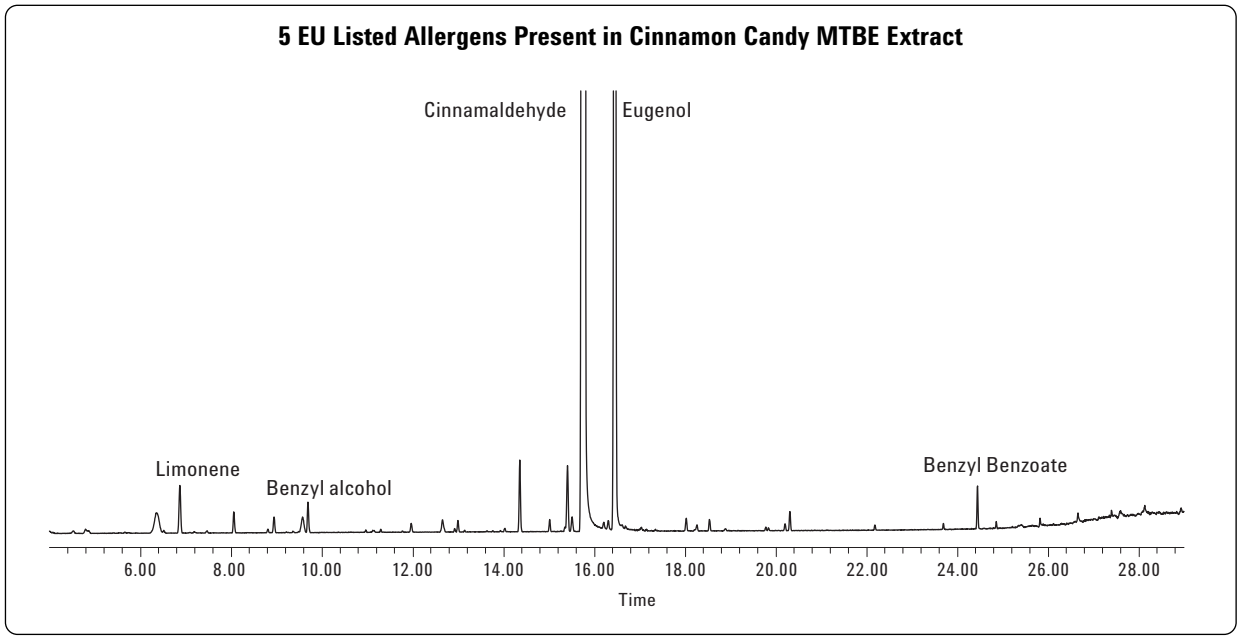

Figure 4. Neat MTBE cinnamon candy extract injection on a Agilent J&W DB-17ms, 30 m × 0.25 mm, 0.25 µm column (p/n 122-4712). GC conditions are in Table 1.

Reference

1. EU Directive 2003/15/EC, *Official Journal of the European Union*, 6 66.26, 11.3.2003
2. 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
3. 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

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