Identification of Flavor and Fragrance Allergens in Some Common Snack Foods Using Transportable Agilent 5975T GC/MS

Application Note

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Abstract

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 transportable Agilent 5975T transportable GC/MS with a Thermal Separation Probe (TSP), this solution is ideally suited for out of lab analysis when fast and timely response is critical

Introduction

The 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 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. The detection of allergens needs fast and timely response to avoid possible injury to the consumer.

Detection 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.

The on-site detection of food additives in mobile lab can save much time for taking sample from field to fixed conventional laboratory. The focus of this application note is a single column Agilent 5975T GC/MS with TSP approach in mobile lab to separate the allergens in flavored candy and gum products using the selectivity offered by a DB-17ms LTM column.

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Experimental

This analysis was done on an Agilent DB-17ms 30 m × 0.25 mm, 0.25 µm LTM column on an Agilent 5975T LTM 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.

Sample Preparation

Individual 1,000 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 1,000 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 seconds 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 minutes on an Eberbach reciprocating shaker set on high speed. Samples were spun down 4,000 times to separate the MTBE and aqueous layers. Samples were taken from the top MTBE layer for analysis.

Instrumentation	**Agilent 5975T LTM GC/MS**
Column	Agilent DB-17 LTM module 30 m × 0.25 mm × 0.25 µm (p/n G3900-63029)
Guard column	0.5 m column with the same phase as the analytical column, connected to the injector
Experimental conditions	
Inlet temperature	250 °C
Injection mode	TSP
Injection mode	split, 50:1
Carrier gas	helium
Constant pressure mode	13.19 psi
LTM Oven	150 °C (0.1 minutes) to 195 °C (7 °C/min); 15 °C/min to 280 °C, 2 minute hold
Isothermal temperature	250 °C
MSD interface	280 °C
Ion source	300 °C
Quad. temperature	180 °C
Ionization mode	EI
Scan mode	full scan, *m/z* 40–500
EMV mode	Gain factor
Gain factor	5.00
Resulting EM voltage	1,400 V
Solvent delay	2.0 minutes

Results and Discussion

Figure 1 shows the separation of 24 GC/MS-amenable allergens on a DB-17ms, 30 m × 0.25 mm, 0.25 µm LTM capillary 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-17 ms 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. TSP would be one way of reducing deleterious matrix effects and increasing sample throughput.

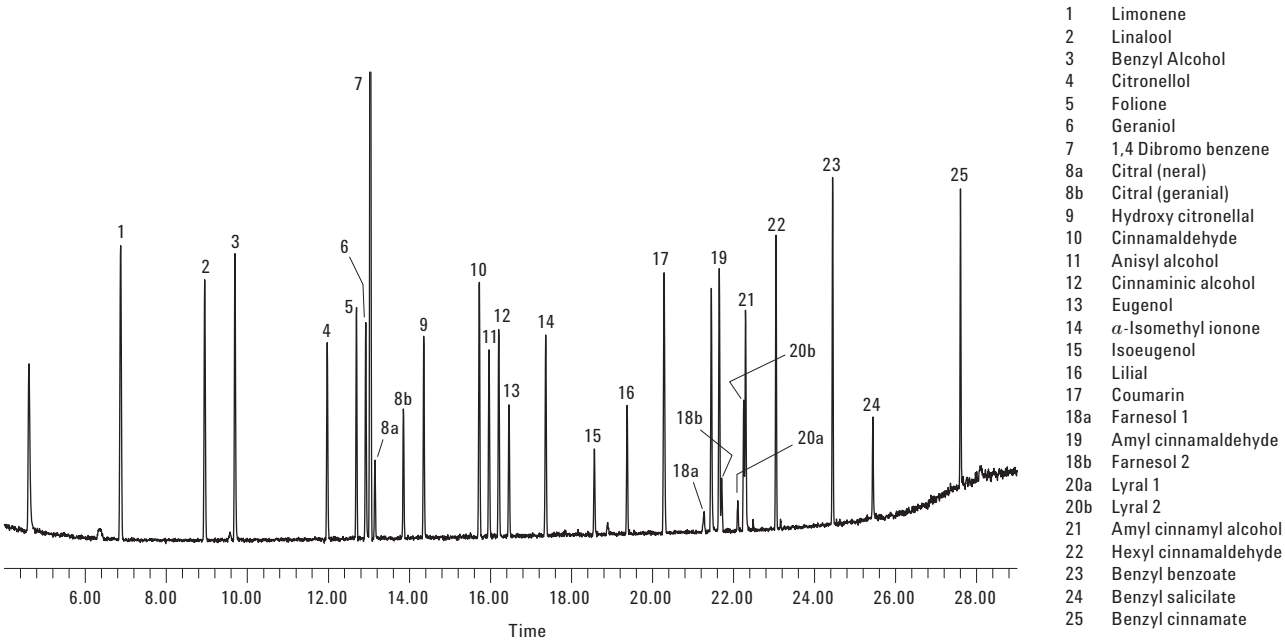

Figure 1. The 8 ppm standard injection of 24 EU allergens on an Agilent DB-17ms, 30 m × 0.25 mm, 0.25 µm LTM column (p/n G3900-63029).

Figure 2 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.

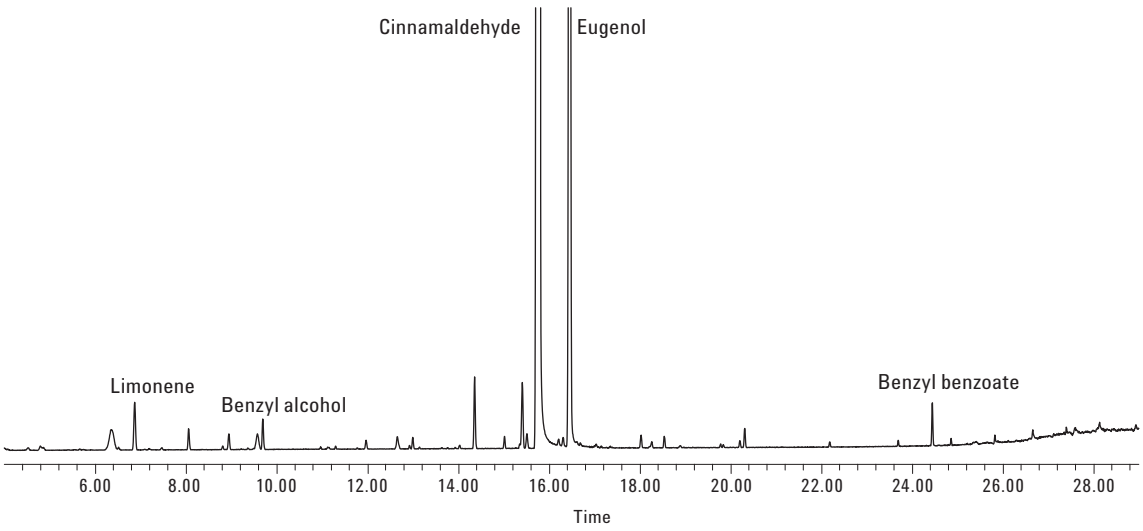

Figure 2. Neat MTBE cinnamon candy extract injection.

Conclusions

This application note successfully demonstrates the utility of Agilent 5975T GC/MS for analysis of the EU flavor and fragrance allergens in candy and gum samples. 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 achieved.

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