Analysis of Suspected Flavor and Fragrance Allergens in Cosmetics Using the 7890A GC and Capillary Column Backflush

Application

Food

Authors

Frank David
Research Institute for Chromatography
Pres. Kennedypark 26
8500 Kortrijk
Belgium

Matthew S. Klee
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Flavor and fragrance allergens are determined in cosmetics using GC-MS. After simple sample preparation by nonselective extraction/dilution, extracts were injected and analyzed under fast screening conditions and locked retention times. After elution of the target solutes, the low-volatility matrix constituents, such as detergents, were effectively removed using capillary column backflush. Column and detector contamination were thereby strongly reduced and sample throughput was significantly increased.

Introduction

According to EU directive 2003/15/EC [1], 27 fragrance compounds in cosmetic products should be labeled if their concentrations exceed 100 ppm (mg/kg) for wash-off products, such as shower gels or soaps, or 10 ppm for leave-on products, such as perfumes or creams. Therefore, qualitative and quantitative methods are needed to monitor these target solutes in various types of cosmetic products.

Depending on the sample matrix and solute concentrations, different sample preparation methods are developed and applied [2]. For the determination of allergens in cosmetic products, one of the major problems is related to the presence of less volatile or nonvolatile constituents, such as detergents (nonionic or ionic), waxes, lipids, etc. These constituents will contaminate the analytical system if the samples are introduced without selective sample preparation. Selective extraction or selective sample introduction is not easy, however, since the target compounds cover a broad volatility range (from limonene to benzyl benzoate) and polarity range (from relatively polar benzyl alcohol to apolar benzyl benzoate). The method of choice should therefore give ppm sensitivity on one hand, and avoid discrimination of the target solutes based on relative volatility or polarity, on the other hand. Moreover, for routine analysis in a quality control environment, sample preparation should be minimized and direct injection of a nonselective solution or extract is preferred. Recently, liquid sample introduction with selective retention of nonvolatiles in a packed PTV liner in combination with automated liner exchange was developed and validated [3]. This approach, however, requires a dedicated sampler.

In this application, an alternative method is proposed using a standard split/splitless inlet and Capillary Flow Technology. A QuickSwap device is used at the end of the column (coupled to the mass spectrometer transfer line), thereby allowing

column outlet pressure to be controlled with auxiliary electronic pneumatic control (EPC). By lowering the inlet pressure and raising the outlet pressure after the last peak of interest has eluted from the column, sample components remaining in the column are forced back out the head of the column into the split inlet and are subsequently trapped on the split vent trap.

The analysis is performed by GC-MS under retention-time locked conditions. The reference method, using a 30 m × 0.25 mm id × 0.25 µm HP-5MS column operated under helium [2], was translated to a fast screening method for maximum throughput, using a 15-m column and hydrogen as the carrier gas. The analysis time needed for the separation of the target solutes was thereby reduced from 24 to 8 minutes (3X speedup). The low-volatility sample matrix constituents are backflushed from the column, avoiding column and detector contamination, baseline shifts, and ghost peaks due to carryover into subsequent runs.

Sample Preparation

Samples are diluted to the 5% level (50 mg/mL) in an appropriate solvent (typically, acetone or dichloromethane is used). The sample is placed in an ultrasonic bath for 15 minutes to completely dissolve the target solutes in the solvent. After extraction and dissolution, the sample can be centrifuged and the supernatant transferred to an autosampler vial.

In this application, data were obtained on a shampoo sample containing fragrance compounds and nonionic detergents.

GC conditions

All analyses were performed on an Agilent 7890A GC-5975 MSD combination. Injection was done using a 7683 ALS. The GC-MS conditions can be summarized as follows:

GC-MS Conditions

Column	15 m x 0.25 mm id x 0.25 µm HP-5MS	Agilent P/N 19091-431
Carrier gas and pressure	Hydrogen	11.050 psi constant pressure
Column outlet and pressure	QuickSwap	4 psi via auxiliary EPC
Inlet	Split/splitless in split mode	250 °C, split ratio = 50:1
Oven temperature program	Fast analysis (3X speedup*)	50 °C (0.33 min) → 240 °C at 24°C/min
MSD setpoints	Transfer line temperature	250 °C
	Source temperature	300 °C
	Quad temperature	150 °C
Tune	Autotune	EMV +0V
QuickSwap restrictor	17 cm x 110 µm id (4 psi)	P/N G3185-60063
Detection	MS in scan mode	40–350 amu, samples = 2[1]
MSD events	Solvent delay	1.5 min
	Detector OFF (during backflush)	8.0 min

* Under these conditions, alpha isomethyl ionone elutes at 5.17 min, corresponding to a speed gain factor of 3 in comparison to a previously published retention time locking (RTL) method [2].

Backflush conditions (initiated at 8 min)

Inlet pressure	2 psi
Auxiliary pressure	70 psi
Backflush time	2.75 min
Backflush temperature	240 °C

Results

First, the shampoo extract was analyzed in a typical mode—without applying backflush and programming the oven to 320 °C to ensure that late eluters were eluted. In Figure 1, the overlay of the total ion chromatograms of 10 consecutive runs is shown. Excellent retention time and peak area repeatability is obtained in the first part of the chromatogram.

In this sample, some allergens could be detected, including limonene (peak 1), linalool (2), eugenol (3), lilial (4), hexyl cinnamaldehyde (5), benzyl benzoate (6), and benzyl salicylate (7). After 8 minutes, no target solutes elute, but peaks corresponding to nonionic detergents are detected. Even using a bakeout at 320 °C, these compounds are not completely removed from the column. This can be seen from the appearance of ghost peaks (for instance, one at 11.7 minutes indicated by an arrow). This peak and others due to carryover increase regularly with added sample injections, clearly indicating that not all low-volatility sample material elutes from the column. Also, an increasing baseline is clearly observed after 10 minutes. It should be noted that from this 14-minute run, only the first 8 minutes are in fact needed for the necessary separation and quantitation of the target allergens. The remaining time represents the common practice of trying to removing highly retained sample components from the column by "baking the column out." As demonstrated here, this is not so easily accomplished.

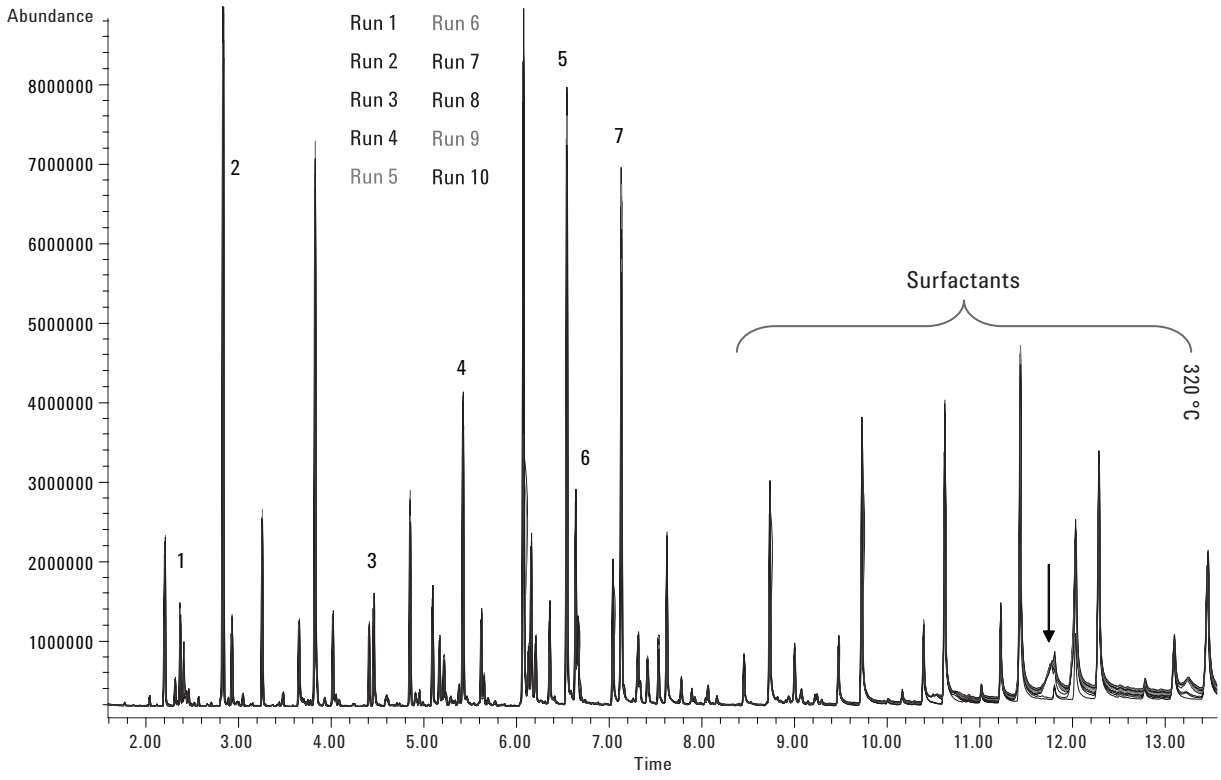

Figure 1. Overlay of 10 consecutive analyses of shampoo extract (oven temperature programmed to 320 °C, no backflush).

After this sequence of 10 sample runs, two blank runs were made. The chromatograms are given in Figure 2. Some contaminant peaks (probably extractables from repeated penetration of sample vial septum) elute around 6 to 8 minutes and are constant in both blank runs. The large peaks, eluting after 10 minutes, clearly show that high molecular weight materials were building up in the column and that these compounds were not removed, even by programming to 320 °C.

In a subsequent experiment, another six consecutive runs of the shampoo extract were made. For each analysis, the run was stopped at 8 minutes after the retention time of the most highly retained target allergen. After the sample runs, two blanks were run: one with the same temperature program as the samples, ending at 240 °C (8 minutes), and another in which the temperature program continued to 320 °C. The chromatograms of the sixth sample analysis, the first blank (stopped at 8 minutes) and the second blank (run to 320 °C) are overlaid in Figure 3.

Some ghost peaks appear within the 8-minute analysis time window, even in the first blank. From the second blank run to 320 °C, it is clear that low-volatility solutes were accumulating in the column from each injected sample. Accumulation of sample material such as that shown in this example quickly leads to column deterioration and greatly reduces the ability to detect and quantify minor sample components. By following the typical approach of attempting to remove late-eluting sample components (cleaning off the column) at high temperature, not only is the column prone to premature degradation due to oxidation and cleavage of stationary phase polymer, but the contamination is moved from the column into the mass spectrometer source, degrading its performance and requiring more frequent cleaning.

Next, a backflush method was set up and 10 new sample runs were made, followed by a blank run. The chromatograms of the sample analyses are shown in Figure 4.

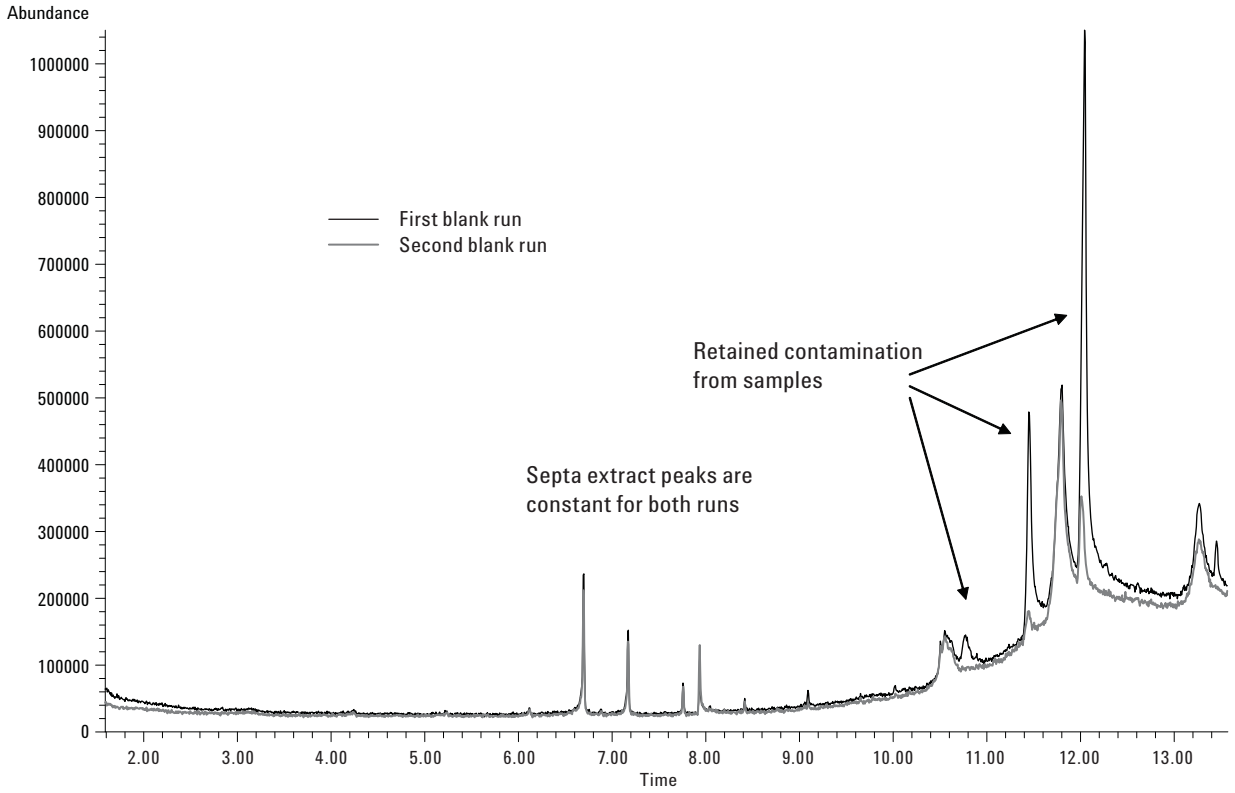

Figure 2. Two consecutive blank runs after analysis of shampoo extract.

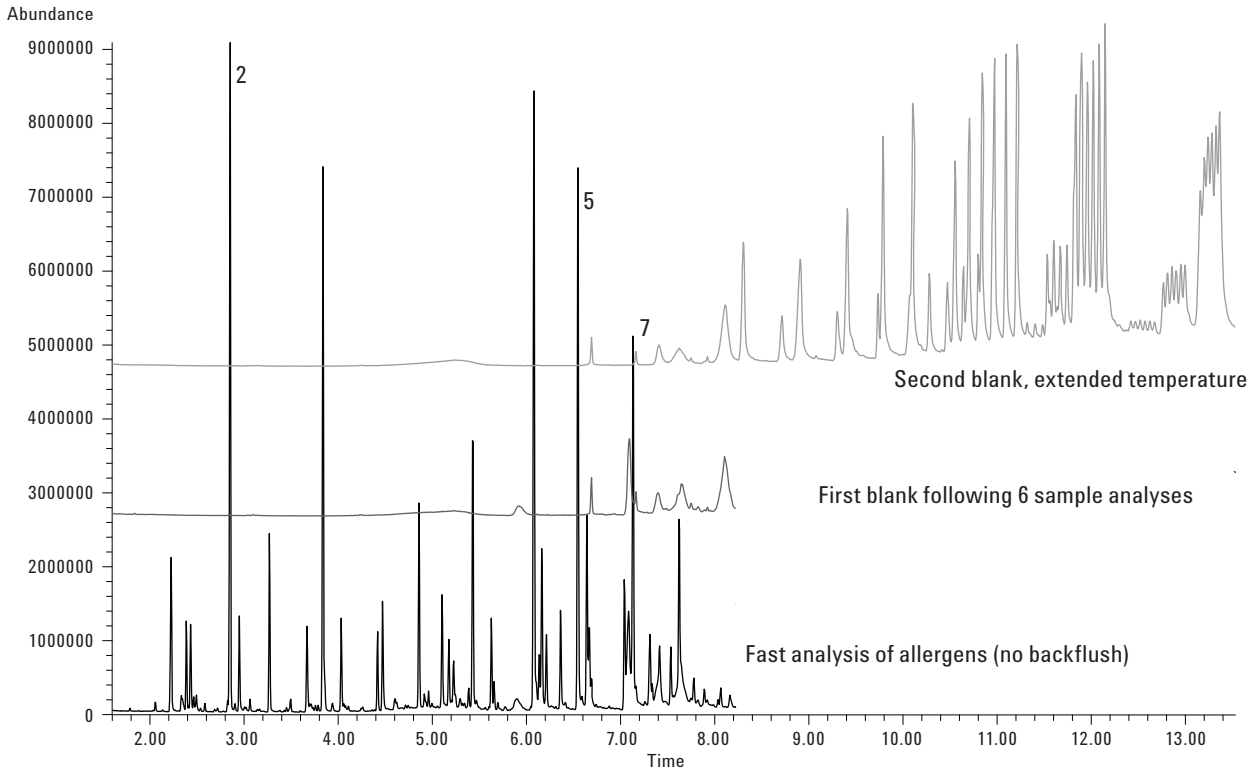

Figure 3. Overlay of sixth analysis of shampoo extract with run stopped at 240 °C (bottom), first blank run to 240 °C (middle), and second blank run to 320 °C (top).

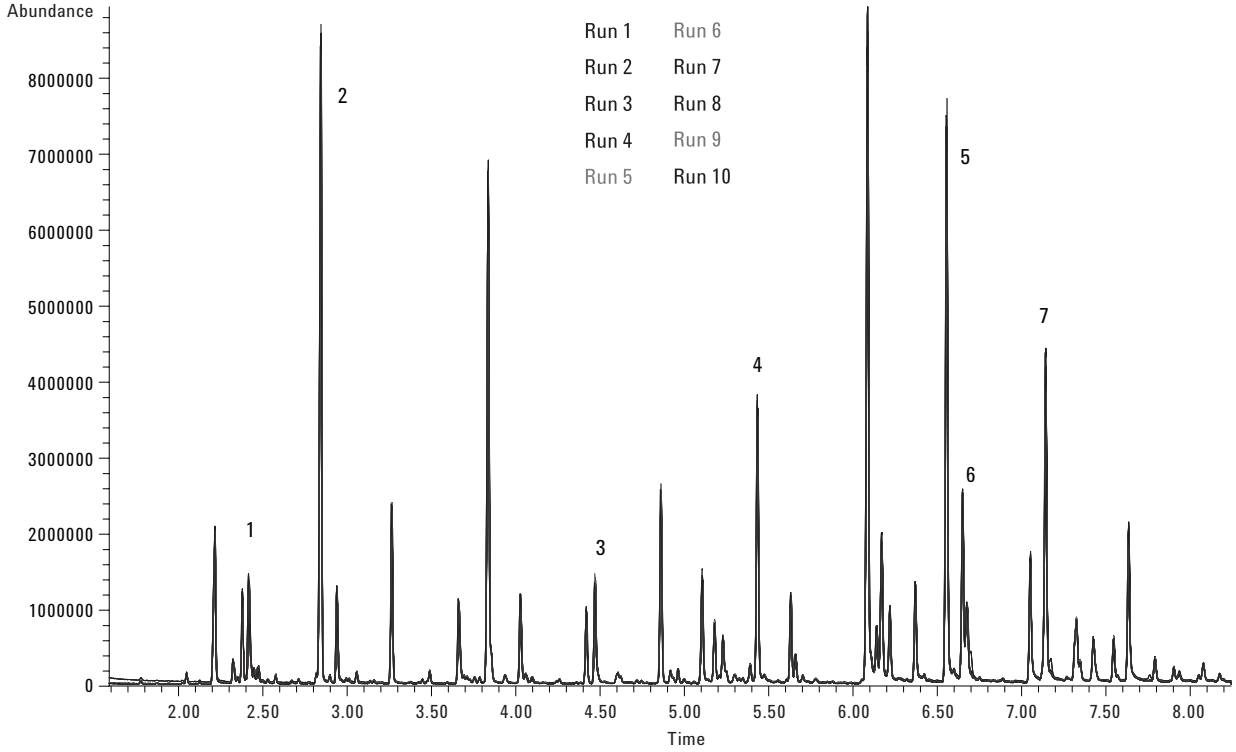

Figure 4. Overlay of 10 consecutive analyses of shampoo extract (oven temperature programmed to 240 °C, with backflush).

From Figure 4, it is clear again that excellent retention time and peak area repeatability were obtained with no evidence of carryover: no emerging ghost peaks; no increasing baseline.

In Figure 5, the tenth run is overlaid with a blank that was run immediately following it. In the blank run, only contaminant peaks coming from the solvent vial septum are observed. The detergent peaks were efficiently and effectively removed from the column.

The total analysis time was reduced from 13.6 min (programmed to 320 °C, with a 2-minute hold) to 11 minutes (programmed to 240 °C, with 2.75-minute backflush). Moreover, all low-volatile material was removed from the column, which was not the case with the longer run without backflush. An added bonus was that the oven cooldown and equilibration times were reduced because of the lower final oven temperature.

Retention time peak area repeatability was determined for each of the seven identified allergens and is listed in Table 1. The standard deviation on the retention times is better than 0.002 minute (RSD < 0.03%). Also, excellent values are obtained for peak area repeatability.

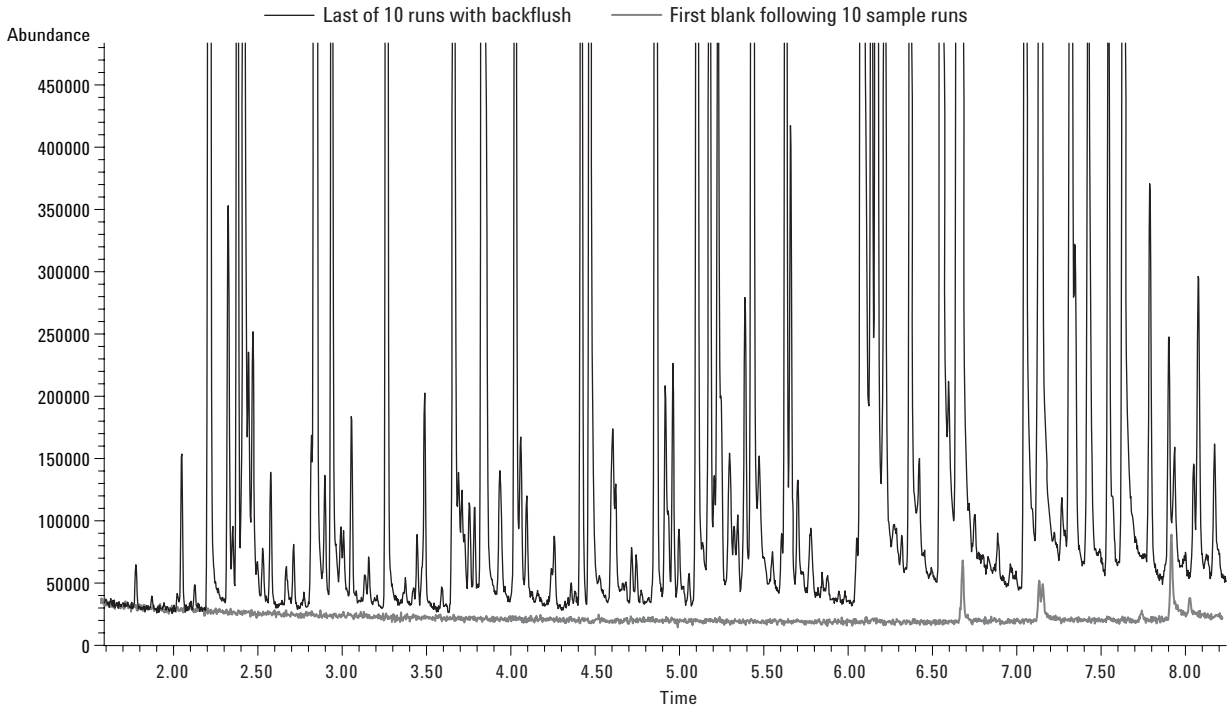

Figure 5. Overlay of 10 analyses of shampoo extract (oven programmed to 240 °C) with backflush (top) and subsequent blank run (bottom).

Table 1. Seven Identified Allergens

	RT min	RT SD min	RT RSD %	Area RSD %
Limonene	2.3771	0.0005	0.020	1.80
Linalool	2.8372	0.0004	0.015%	1.60
Eugenol	4.4671	0.0003	0.007	1.60
Lilial	5.4312	0.0015	0.028	1.53
Hexyl cinnamaldehyde	6.5514	0.0016	0.022	2.00
Benzyl benzoate	6.6467	0.0000	0.000	2.00
Benzyl salicylate	7.1405	0.0013	0.018	2.98
Average		**0.0008**	**0.015**	**1.95**

Conclusions

For the determination of flavor and fragrance allergens in cosmetics, direct sample injection in a split/splitless inlet can be used. In comparison to a previously presented retention time locked method, the analysis time was reduced by a factor of three using a shorter column and hydrogen as carrier gas in combination with 5975 MSD. Contamination of the column and detector was minimized using the backflush method with the 7890A GC. A 20% reduction of the run time is obtained, with faster oven recycle times. Ghost peaks from previous injections were eliminated. Excellent retention time repeatability and peak area repeatability were obtained.

Since the analysis of flavor and fragrance compounds is also performed on columns with a polar stationary phase and limited maximum operating temperature, for example, polyethylene glycol columns (MAOT 250 °C), the capillary column backflush technique using Capillary Flow Technology with the 7890A GC is a very interesting tool to remove highly retained sample components at moderate temperatures.

References

1. Directive 2003/15/EC, Official Journal of the European Union, L 66/26, 11.3.2003.
2. F. David, C. Devos, and P. Sandra, LC. GC Europe, 19, 602-616, November 2005.
3. F. David, C. Devos, D. Joulain, A. Chaintreau, and P. Sandra, *J. Sep. Science*, 29, 1587-1594 (2006).

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