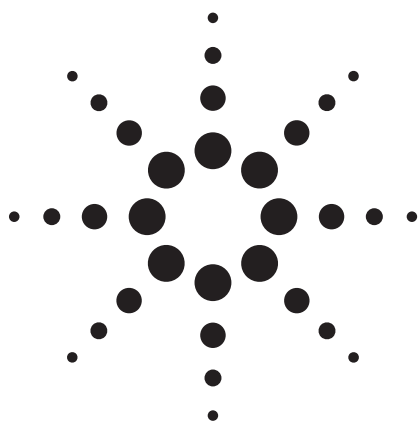# Determination of Allergens in Fragrance Products Using Agilent Deconvolution Reporting Software

Application Brief

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Flavor and Fragrance

The 7th amendment of the European Cosmetics Directive (2003/15/EC) was published in 2003. Manufacturers of cosmetics were required to indicate the presence of 26 fragrance ingredients in finished cosmetic products if they exceed a threshold of 0.01% for rinse-off and 0.001% for leave-on products. Subsequently, the analytical method for 24 allergenic compounds (except two natural extracts – oak moss and tree moss) using GC/MS was published by the International Fragrance Association (IFRA). Natural cosmetics are very complex, containing sterols, waxes, and flavanoids, which can result in poor spectral matches and long data processing time and can generate potential false positive or negative results.

Agilent's deconvolution reporting software (DRS) is designed to automatically deconvolute the spectra from matrices and generate a qualification and quantitation report. DRS integrates information from three processes: MSD ChemStation, automated mass spectral deconvolution and identification system (AMDIS), and NIST Search. DRS increases the confidence in results with complex matrices, and the typical data processing time is about 2 to 3 minutes.

A DRS database of 24 regulated allergenic compounds has been developed (Table 1). Additional compounds can easily be added to the database by the user.

Highlights

- Automated deconvolution increases productivity for analysis of complex matrices

- Allergens database is available as a free download from Agilent Technologies, Inc.

Table 1. Allergens in Fragrance Products

Locked RT	Name	CAS no	Mol form	Mol wt
7.10	Limonene	5989-27-5	$C_{10}H_{16}$	136.1
7.17	Benzyl alcohol	100-51-6	C_7H_8O	108.1
7.41	Phenyl acetaldehyde	122-78-1	$C_8H_8O_2$	120.1
8.47	Linalol	78-70-6	$C_{10}H_{18}O$	154.1
10.29	1,4-Dibromobenzene [ISTD]	106-37-6	$C_6H_4Br_2$	233.9
10.41	Estragole	140-67-0	$C_{10}H_{12}O$	148.2
10.47	Folione	111-12-6	$C_9H_{14}O_2$	154.1
10.94	Citronellol	106-22-9	$C_{10}H_{20}O$	156.2
11.23	Citral (Neral)	106-26-3	$C_{10}H_{16}O$	152.1
11.44	Geraniol	106-24-1	$C_{10}H_{18}O$	154.1
11.77	Citral (geranial)	5392-40-5	$C_{10}H_{16}O$	152.1
11.80	Cinnamaldehyde	104-55-2	C_9H_8O	132.1

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Table 1. Allergens in Fragrance Products (Continued)

Locked RT	Name	CAS no	Mol form	Mol wt
12.00	Anisyl alcohol	105-13-5	$C_8H_{10}O_2$	138.1
12.04	Hydroxy citronellal	107-75-5	$C_{10}H_{20}O_2$	172.2
12.33	Methyl octine carbonate	111-80-8	$C_{10}H_{16}O_2$	168.1
12.42	Cinnamic alcohol	104-54-1	$C_9H_{10}O$	134.1
13.37	Eugenol	97-53-0	$C_{10}H_{12}O_2$	164.1
14.13	Methyl eugenol	93-15-2	$C_{11}H_{14}O_2$	178.2
14.82	Coumarin	91-64-5	$C_9H_6O_2$	146.0
14.88	Cinnamyl acetate	103-548	$C_{11}H_{12}O_2$	176.1
14.96	Isoeugenol	97-54-1	$C_{10}H_{12}O_2$	164.1
15.5	Alpha isomethyl ionone	127-51-5	$C_{14}H_{22}O$	206.2
16.26	Lilial	80-54-6	$C_{14}H_{20}O$	204.2
18.14	Amyl cinnamaldehyde	122-40-7	$C_{14}H_{18}O$	202.1
18.27	Lyral 1	31906-04-5	$C_{13}H_{22}O_2$	210.2
18.36	Lyral 2	31906-04-4	$C_{13}H_{22}O_2$	210.2
18.7	Amyl cinnamyl alcohol	101-85-9	$C_{14}H_{20}O$	204.2
18.83	Farnesol 1	100009-91-0	$C_{15}H_{26}O$	222.2
19.18	Farnesol 2	4602-84-0	$C_{15}H_{26}O$	222.2
19.61	Hexyl cinnamaldehyde	101-86-0	$C_{15}H_{20}O$	216.2
19.89	Benzyl benzoate	120-51-4	$C_{14}H_{12}O_2$	212.1
21.36	Benzyl salicylate	118-58-1	$C_{14}H_{12}O_3$	228.1
24.20	Benzyl cinnamate	103-41-3	$C_{16}H_{14}O_2$	238.1

The Agilent Retention Time Locked (RTL) database was developed using the instrument conditions in Table 2, which is locked to alpha-isomethyl ionone in 15.494 min by retention time locking.

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

GC	Agilent Technologies 7890A or 6890N		
Back inlet	Split/splitless		
Injection type	Split		
Inlet temperature	250 °C		
Pressure	11.46 psi		
Split ratio	50:1		
Split flow	68.5 mL/min		
Total flow	72.7 mL/min		
Gas saver	On		
Saver flow	15.0 mL/min		
Saver time	1.00 min		
Gas type	Helium		
4 mm ID, Single Taper Liner			
Oven			
Oven ramp	°C/min	Next °C	Hold min
Initial time		50	1
Ramp rate	8	270	1.5
Total run time	30 min		
Equilibration time	0.5 min		
Column	Agilent Technologies HP-5ms p/n 19091S-433		
Length	30 m		
Diameter	0.25 mm		
Film thickness	0.25 µm		
Mode	Constant pressure		
Pressure	11.46 psi		
Nominal initial flow	1.4 mL/min		
Inlet	Back inlet		
Outlet	MSD		
Outlet pressure	Vacuum		

Table 2. Gas Chromatograph and Mass Spectrometer Conditions (Continued)

RTL	System retention time locked to alpha isomethyl ionone at 15.494 min
Back Injector	
Sample washes	1
Sample pumps	3
Injection volume	1 µL
Syringe size	10 µL
Preinj solvent A washes	3
Preinj solvent B washes	3
Postinj solvent A washes	3
Postinj solvent B washes	3
Viscosity delay	1 second
Plunger speed	Fast
Preinjection dwell	0 minutes
Post-injection dwell	0 minutes
MSD	Agilent Technologies 5975C
Acquistion mode	Scan/SIM
Solvent delay	3 min
Low mass	40
High mass	350
Threshold	20
Sampling	3
Quad temperature	150 °C
Source temperature	230 °C
Transfer line temperature	280 °C
Tune type	Autotune
EM voltage	Atune voltage, 1,023.5 V
MSD-SIM	AutoSIM was used to pick ions, groups, and switching times
Number of groups	17
Ions/group	Varied 4 to 14
Dwell time, msec	10
Cycles/peak	Varied 5.8 to 15.9

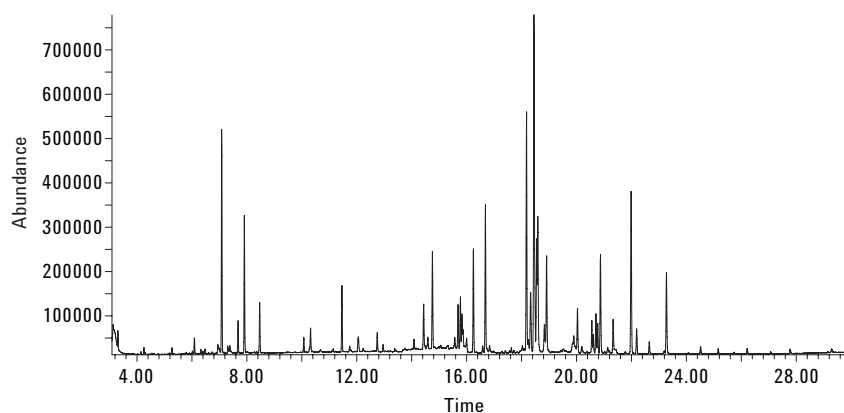A typical total ion chromatogram (TIC) of a fragrance product is displayed in Figure 1.

Figure 1. Total ion chromatogram of a fragrance product.

Dirty matrices often disturb the identification of target compounds by high-level chemical noise, resulting in poor library match factors. Background subtraction is both matrix- and operator-dependent and can yield inconsistent results. DRS automatically deconvolutes the signal from the matrix using AMDIS, which identifies target allergens quickly while minimizing false positives and negatives. A typical DRS report is displayed in Figure 2.

MSD Deconvolution Report
Sample Name: Lilac fragrance 10.20mg
Data File: C:\msdchem\1\DATA\Lilac.D
Date/Time: 10:49 AM Thursday, May 8 2008

Adjacent Peak Subtraction = 1
Resolution = Medium
Sensitivity = Medium
Shape Requirements = Medium

The NIST library was searched for the components that were found in the AMDIS target library.

			Amount (ng)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Num.
7.0984	5989275	Limonene	0.01		97	-0.1	94	1
7.1792	100516	Benzyl alcohol			84	0.7	86	1
7.3942	122781	Phenyl acetaldehyde			90	-0.3	94	1
8.4788	78706	Linalol	0.09		96	0.7	91	1
12.105	107755	Hydroxy citronellal	0.54		87	4.3	85	1
12.436	104541	Cinnamic alcohol	0.01		89	1.2	76	1
12.4495	111808	Methyl octine carbonate			56	8.0		
12.4495	22771444	cis-p-Mentha-2,8-dien-1-ol					71	1
14.8775	91645	Coumarin			91	3.7	92	1
15.1775	103548	Cinnamyl acetate			44	18.6		
15.1775	103957	3-(4-Isopropylphenyl)-2-methylpropionaldehyde					89	1
18.1555	122407	Amyl cinnamaldehyde			99	1.3	93	1
19.6005	101860	Hexyl cinnamaldehyde			94	0.0	89	1
19.8882	120514	Benzyl benzoate	0.01		72	0.3	91	1
24.1972	103413	Benzyl cinnamate			96	-0.2	90	2
10.296		1,4 Dibromobenzene	1					

Figure 2.   Typical DRS report.

Figure 3 shows us the benefit of DRS when analyzing allergens in fragrance products. Cinnamaldehyde was successfully identified by DRS even though it was buried by the coeluting matrix compounds. The upper window is the TIC, the middle window is the raw or dirty spectrum in the scan No. 987 (11.796 min), and the lower window is the comparison of the deconvoluted spectrum (the white plot) with the spectrum of cinnamaldehyde in the allergen RTL library (the black plot). After deconvolution, the spectrum of the scan No. 987 is "clean," and we can easily identify the cinnamaldehyde in the fragrance product.

If your laboratory already has a user library in Agilent format, DRS A.04 software can create the necessary files in AMDIS format for deconvolution. Your laboratory may also have an optimized method or preferred column for allergens analysis. In these cases, DRS A.04 software can still be used with your own retention times and method.
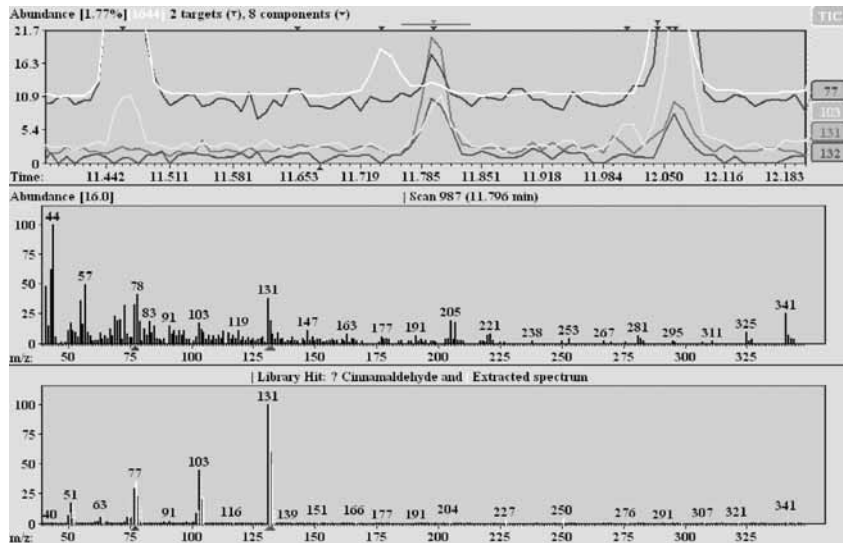
Figure 3.   AMDIS display showing: a) the total ion chromatogram of a fragrance
            product; b) the spectrum where cinnamaldehyde elutes, and c) the decon-
            voluted spectrum (in white) juxtaposed to the library spectrum for
            cinnamaldehyde (in black).

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Summary

DRS (p/n G1716AA) automatically deconvolutes mass spectra and produces more consistent and reliable identification of compounds in complex matrices. A DRS add-on allergen RTL database has been published on the Agilent Web site and, after registration, can be freely downloaded from http://www.chem .agilent.com/en-US/Support/Downloads/Utilities/RetentionTimeLocking/ Pages/default.aspx.

References

1. "GC/MS Quantitation of Potential Fragrance Allergens in Fragrance Compounds," Version 3, September 12, 2007, International Fragrance Association

2. "Data Treatment Strategy to Analyze Allergens in Complex Samples Using the IFRA Procedure," Version 3, September 12, 2007, IInternational Fragrance Association

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