Quantitative Analysis of Allergens in Perfumes Using Comprehensive Two-Dimensional GC and Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

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Key Words: GCxGC-TOFMS, Food and Flavors, Quantitation

1. Introduction

Fragrances are complex mixtures that are part of everyday life. From cosmetics to household products, chemicals that produce scent are present at various levels, but little is known about their composition since fragrance formulas are considered trade secrets. New health concerns about allergic reactions caused by chemicals present in fragrances of synthetic or natural origin (it is estimated that 1 to 2% of the population has allergies to fragrance) have led to an increased interest in the analysis of perfumes. The 7th Amendment of the European Cosmetics Directive requires the declaration of the listed 26 "fragrance allergens" (24 defined volatile substances and 2 botanicals) if they exceed specified levels. As a result, a project was initiated at the end of 2001 within the International Fragrance Association (IFRA) to develop a method for the analysis of "fragrance allergens", with the purpose of defining a way to determine and regulate their presence.

Comprehensive two-dimensional gas chromatography (GCxGC) provides the additional peak capacity necessary to elucidate the composition of complex sample mixtures. Two separation mechanisms (primary and secondary columns of different phases) are employed to aid in the analysis of such complex samples. With thermal modulation (after the primary column), peak widths at the end of the second column are typically on the order of 100 ms, and fast data acquisition systems are required. A Time-of-Flight Mass Spectrometer (TOFMS) is the only MS capable of acquisition rates up to 500 spectra/second, adequate for the characterization of peaks with low ms widths.

For this study of allergens in a perfume, two different secondary columns were tested (DB-WAX and VB-210) while the first column was kept as a constant (Rtx-5MS).

This study is concerned with the detection in perfumes of 24-targeted compounds and 3 additional volatiles with other regulatory concerns. A GCxGC-TOFMS system is used to find, identify, and quantify the presence of all components in one analysis.

2. Experimental Conditions

GCxGC:
Agilent 6890 GC equipped with a LECO Thermal Modulator (technology under license from Zoex Corporation)
Primary Column:
Rtx-5, 30 m, 0.25 mm id, 0.25 mm film thickness
Carrier Gas:
He at a constant flow of 1.5 ml/minute
Inlet Temperature: 250°C
Injection Size: 1 ml
Split Ratio: 100:1
MS: LECO Pegasus® TOFMS
Ionization: EI at 70eV
Mass Range (u): 35 to 350
Acquisition Rate: 150 spectra/second

All other parameters are listed in Table 1 as a function of column type used in the second dimension.

Table 1. GCxGC experimental conditions.

Column (type and dimensions)	VB-210, 2 m, 0.1 mm x 0.1 µm	DB-WAX, 1 m, 0.1 mm x 0.1 µm
Main Oven Program	90°C (hold 1 min) to 300°C at 10°C/min	90°C (hold 1 min) to 235°C at 5°C/min
Modulator Temperature Offset from Main Oven (°C)	30	30
Modulator Frequency (seconds)	4	5
Hot Pulse Duration (seconds)	0.5	0.8
Secondary Oven Program	95°C (hold 1 min) to 300°C at 10°C/min	100°C (hold 1 min) to 23 5°C at 5°C/min
Transfer Line Temperature (°C)	250	200
Source Temperature (°C)	250	200

3. Results

Qualitative Analysis

In addition to the 24 targeted allergenic compounds, the standard mixture included 11 additional components.

- 2 internal standards to be used for quantification
- 5 impurities (isomers of the targeted compounds) present in the standard used for the preparation of the mixture
- 3 carcinogenic compounds
- 1 component (benzeneacetaldehyde) sought to be added on the targeted allergens list

All 35 components present in the standard mixture along with their retention times (Rtx-5/DB-WAX) and unique masses are listed in Table 2.

Figures 1 and 3 show the total ion current (TIC) chromatograms as contour plots for the two different column configurations. Peak intensity is color scaled in the figures from blue to red, with red representing the highest intensity.

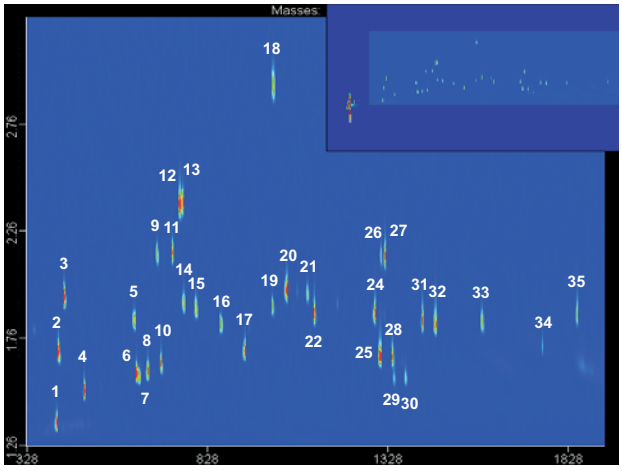

Figure 1. TIC chromatogram of the standard mixture on the column set Rtx-5/VB-210. The chromatogram on the upper right corner is the full-scale chromatogram with the highlighted region representing the zoomed display. Peak numbers are the same as in Table 2.

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Table 2. Peak numbers, retention times (Rtx-5/DB-WAX), and unique masses for all standard components.

Peak #	Name	Retention Time (seconds) 1st Dimension	2nd Dimension	Unique Mass
1	Limonene	350	0.57	68
2	Benzyl alcohol	355	2.74	106
3	Benzeneacetaldehyde	365	1.27	91
4	Linalool	420	0.91	71
5	Methyl heptine carbonate	550	0.95	67
6	Estragole[3]	555	1.00	148
7	*1,4-dibromobenzene*[1]	555	1.15	236
8	Citronellol	585	1.17	69
9	Neral	605	0.97	69
10	Geraniol	620	1.34	69
11	Geranial	650	1.00	69
12	Cinnamaldehyde	665	2.27	131
13	Hydroxycitronellal	675	1.55	59
14	Anisyl alcohol	675	4.05	138
15	Cinnamic alcohol	710	3.69	92
16	Eugenol	775	2.19	164
17	Methyl eugenol[3]	840	1.31	178
18	Coumarin	910	3.21	118
19	Isoeugenol	915	2.49	164
20	à Isomethyl ionone	950	0.87	135
21	à-N-Methyl ionone[2]	1005	0.89	121
22	Lilial[3]	1025	1.07	189
23	à-Methylionone[2]	1085	0.93	191
24	Amylcinnamic aldehyde	1185	1.21	115
25	8-Phenyl-1-octanol[2]	1200	1.56	91
26	Lyral 1[2]	1205	1.74	59
27	Lyral 2	1215	1.76	79
28	Amyl cinnamic alcohol	1235	1.88	91
29	Farnesol 1	1240	1.23	69
30	Farnesol 2[2]	1275	1.22	69
31	Hexylcinnamic aldehyde	1315	1.19	115
32	Benzyl benzoate	1350	1.80	105
33	Benzyl salicylate	1480	1.88	91
34	*4,4'-dibromo-1,1'-biphenyl*[1]	1640	1.95	152
35	Benzyl cinnamate	1735	2.28	91

[1] *Internal standards*
[2] *Impurities found in the standard mixture*
[3] *Carcinogenic compounds*

When the Rtx-5/VB-210 column set was used, peak 23 was not detected in either the standard mixture or the spiked perfume sample. Even though the rest of the components were separated when the standard mixture was analyzed, interference with the matrix caused poor quantitative results for the spiked perfume sample. The 34 components eluted in a time frame of only 1.58 seconds in the second dimension. A more polar column that can produce an increased separation in the second dimension seemed to be needed in order to reduce matrix interference.

Even with the great increase in peak capacity obtained by GCxGC, coelutions of peaks can still be present. The TOFMS has the advantage of spectral continuity across the chromatographic peak profile (no spectral skewing) that allows the deconvolution algorithm to correctly extract accurate spectral information for coeluting peaks. An example of deconvolution with spectral data for the deconvoluted peaks, as well as for the NIST library hit, is presented in Figure 2. Even though peak apex separation is less than 50 ms, similarities with the library are above 900 (1000 being the perfect match).

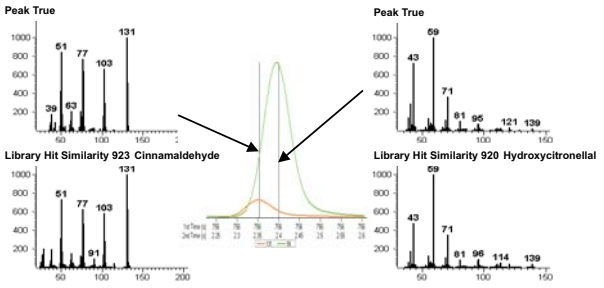

Figure 2. Deconvolution results for peaks 12 and 13 when the Rtx-5/VB-210 column set was used. Deconvoluted mass spectral data and NIST library hits are presented for both analytes.

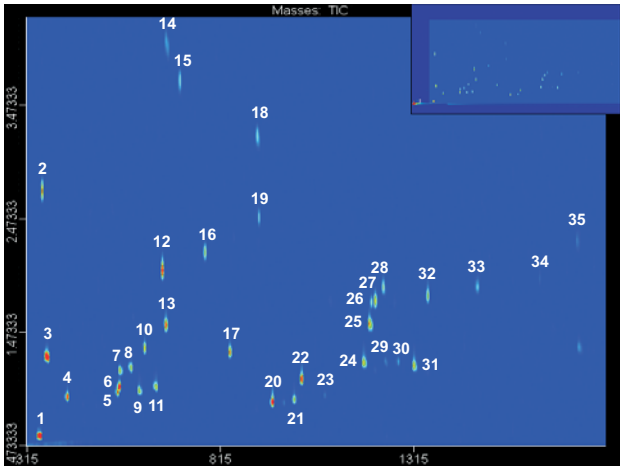

Figure 3. TIC chromatogram of the standard mixture on the column set Rtx-5/DB-WAX. The chromatogram on the upper right corner is the full-scale chromatogram with the highlighted region representing the zoomed display. Peak numbers are the same as in Table 2.

The Rtx-5/DB-WAX column combination provided the best overall separation of the 35 components. Their retention times in the second dimension ranged from 0.57 seconds to 4.05 seconds for a total 2nd dimension separation time of almost 3.5 seconds. Component 23 was now well separated and detected in both the standard mixture and a spiked sample. Matrix interference was reduced and good quantification results were obtained when a spiked perfume sample was analyzed.

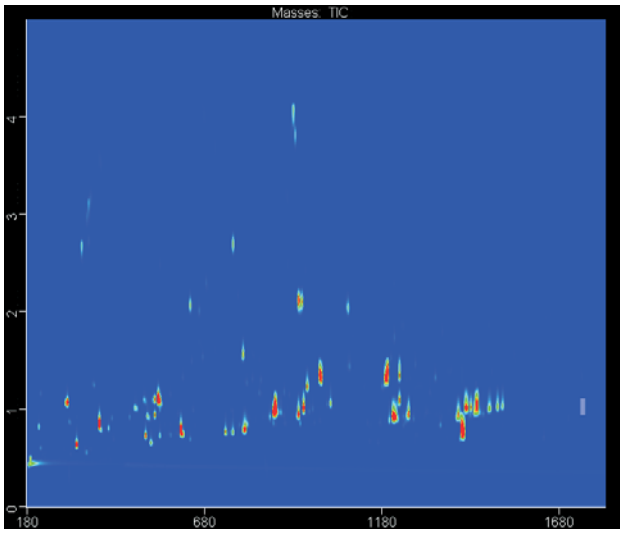

Figure 4. TIC chromatogram of a spiked perfume sample. The analysis was performed on the Rtx-5/DB-WAX column set.

Figure 4 represents the TIC chromatogram of the spiked perfume sample on the Rtx-5/DB-WAX column set. The increase in separation power in the second dimension obtained from the DB-WAX column resulted in good separation of all the standard analytes from the matrix components. The TIC chromatogram, as well as the extracted ion chromatogram for a smaller group of compounds, is presented in Figure 5.

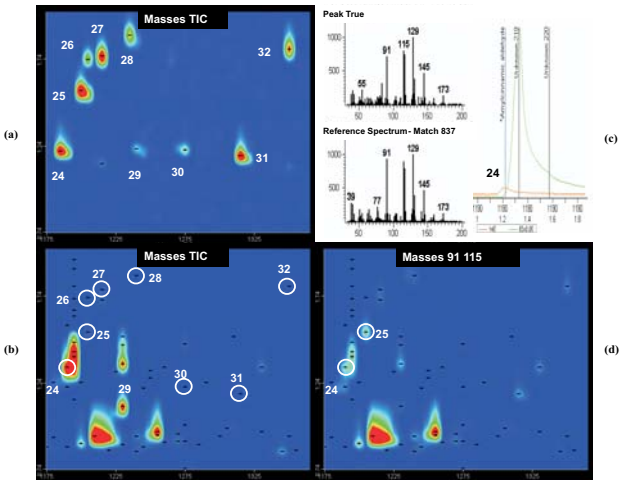

Figure 5. TIC chromatograms for the standard mixture (a) and the spiked perfume sample (b). Unique ions for amylcinnamic aldehyde (peak 24) and 8-phenyl-1-octanol (peak 25) are plotted for the spiked sample (d). Mass spectral data after deconvolution are presented for amylcinnamic aldehyde in part (c) of the figure (note the m/z 83 was multiplied by 0.05).

Plots of unique ions for components of interest make these analytes more easily observed than when the TIC chromatogram is displayed. They also make it easier to review automated peak finding and identification obtained from the LECO ChromaTOF software used for data processing. In part (b) of Figure 5, peak 24 is almost completely masked by matrix, while peak 25 and most of the other allergens are lost in the blue background due to the big concentration difference between the analyte of interest and the matrix. When m/z 91 and 115 are plotted, the two peaks of interest are more easily observed. Part (c) of the figure shows very good deconvolution results for peak 24 (match of 837 with the reference standard) even though its intensity is 56 times smaller than for the matrix peak and the separation between peak apexes is only 100 ms.

Quantitative Analysis

Standards at five different concentration levels ranging from around 20 to 300 ppm were used to construct the calibration curves. Correlation coefficients (r) were above 0.987 for all 33 analytes. Two internal standards were used and the results are presented in Table 3. Examples of calibration curves are presented in Figures 6 and 7.

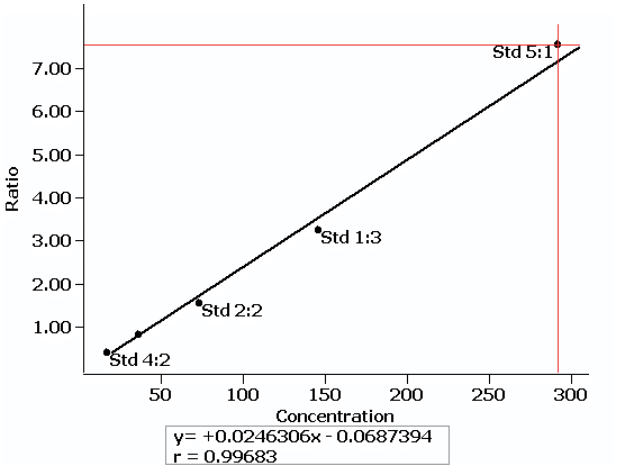

Figure 6. Calibration curve for limonene for a concentration range of 19.6 ppm to 307.3 ppm.

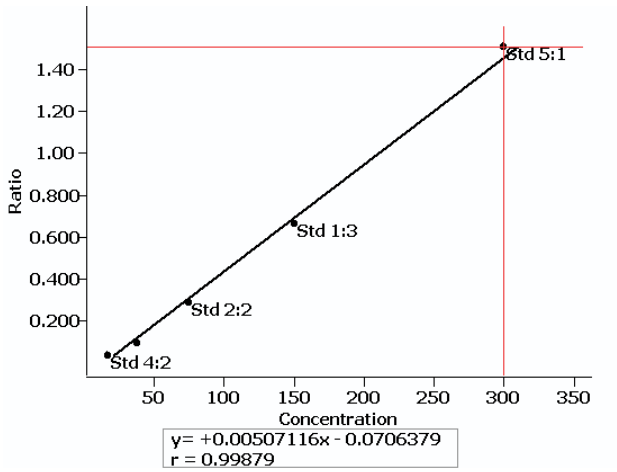

Figure 7. Calibration curve for Isoeugenol for a concentration range of 20.9 ppm to 310 ppm.

Table 3. Correlation coefficients and quantification masses for the 33 analytes present in the standard mixture.

Name	Quant Mass	Internal Standard	r
Limonene	93	1,4-dibromobenzene	0.997
Benzyl alcohol	79	1,4-dibromobenzene	0.997
Benzeneacetaldehyde	91	1,4-dibromobenzene	0.993
Linalool	93	1,4-dibromobenzene	0.999
Methyl heptine carbonate	67	1,4-dibromobenzene	0.999
Estragole	148	1,4-dibromobenzene	0.994
Citronellol	156+138	1,4-dibromobenzene	0.993
Neral	41	1,4-dibromobenzene	0.995
Geraniol	123	1,4-dibromobenzene	0.998
Geranial	69	1,4-dibromobenzene	0.995
Cinnamaldehyde	131	1,4-dibromobenzene	0.987
Hydroxycitronellal	71	1,4-dibromobenzene	0.994
Anisyl alcohol	138	1,4-dibromobenzene	0.999
Cinnamic alcohol	92	1,4-dibromobenzene	0.998
Eugenol	164	1,4-dibromobenzene	0.998
Methyl eugenol	178	1,4-dibromobenzene	0.993
Coumarin	118	1,4-dibromobenzene	0.994
Isoeugenol	164	1,4-dibromobenzene	0.999
à Isomethyl ionone	135	1,4-dibromobenzene	0.998
à-N-Methyl ionone	121	1,4-dibromobenzene	0.998
Lilial	189	1,4-dibromobenzene	0.995
à-Methylionone	191	1,4-dibromobenzene	0.991
Amylcinnamic aldehyde	115	4,4'-dibromo-1, 1'-biphenyl	0.991
8-Phenyl-1-octanol	91	4,4'-dibromo-1, 1'-biphenyl	0.998
Lyral 1	105	4,4'-dibromo-1, 1'-biphenyl	0.990
Lyral 2	136	4,4'-dibromo-1, 1'-biphenyl	0.993
Amylcinnamic alcohol	133	4,4'-dibromo-1, 1'-biphenyl	0.999
Farnesol 1	69	4,4'-dibromo-1, 1'-biphenyl	0.994
Farnesol 2	69	4,4'-dibromo-1, 1'-biphenyl	0.999
Hexylcinnamic aldehyde	129	4,4'-dibromo-1, 1'-biphenyl	0.992
Benzyl benzoate	105	4,4'-dibromo-1, 1'-biphenyl	1.000
Benzyl salicylate	91	4,4'-dibromo-1, 1'-biphenyl	1.000
Benzyl cinnamate	103	4,4'-dibromo-1, 1'-biphenyl	0.998

Delivering the Right Results

Masses used for quantification for all the analytes were chosen as a compromise between abundance in the mass spectrum and uniqueness from the coeluting analytes. After the calibration curves were built, a perfume sample spiked with about 77 ppm of the standard mixture, and an unspiked sample were quantified using the calibration curves. Results are presented in Table 4. With the exception of estragole, the iso-methylionone isomers, and benzeneacetaldehyde (these components were shown to be unstable in previously published literature), all analytes had recoveries within 20% (16 ppm) of the expected values.

Table 4. Recovery results for spiked perfume sample.

	Concentration (ppm)			
Analyte Name	**Unspiked Sample**	**Spiking Amount**	**Spiked Sample (determined)**	**% Recovery**
Limonene	18	73	92	99
Benzyl alcohol	2	79	85	95
Benzeneacetaldehyde	0	78	61	127
Linalool	11	75	93	93
Methyl heptine carbonate	5	80	78	109
Estragole	0	77	109	71
Citronellol	1	74	72	104
Neral	9	75	71	118
Geraniol	0	79	92	86
Geranial	8	75	81	102
Cinnamaldehyde	0	77	64	120
Hydroxycitronellal	0	78	69	112
Anisyl alcohol	0	80	90	89
Cinnamic alcohol	0	73	70	104
Eugenol	0	83	83	100
Methyl eugenol	0	75	87	87
Coumarin	12	76	80	110
Isoeugenol	0	75	83	90
à Isomethyl ionone	4	70	126	59
à-N-Methyl ionone[2]	6	70	99	77
Lilial	6	75	102	79
à-Methylionone	13	70	88	95
Amylcinnamic aldehyde	0	76	65	117
8-Phenyl-1-octanol	0	98	91	107
Lyral 1	0	78	68	115
Lyral 2	30	78	108	100
Amyl cinnamic alcohol	0	98	113	86
Farnesol 1	0	74	75	99
Farnesol 2	0	74	61	121
Hexylcinnamic aldehyde	0	73	66	110
Benzyl benzoate	0	78	88	89
Benzyl salicylate	0	81	71	114
Benzyl cinnamate	0	74	72	103

4. Conclusions

Thirty-three targeted compounds and two internal standards were used to obtain calibration curves with the Pegasus 4D GCxGC-TOFMS system. Different column combinations were tested and it was determined that Rtx-5/DB-WAX gave the best results in separating the analytes from the matrix interferences. One spiked sample was then analyzed to determine recoveries of the analytes in a perfume sample. The spiked sample showed good recoveries for all analytes with only a few exceptions. The compounds with poor recovery were described as unstable in previously published literature and are not part of the 24 volatiles present on the 7th Amendment of the European Cosmetics Directive list.

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