

Determination of 59 potential Allergens in Perfumes by twin-line fast GCMSMS

▪ Introduction

Several chemicals in fragrance products like perfumes or shower gels can cause allergic reaction. These compounds are defined as potential allergens and 24 chemicals plus 2 isomers were regulated by the EU.^[1] Cosmetic products are subdivided into leave on (e.g. perfumes, cremes) or rinse off (shower gels, soaps etc.). The analytical method was using GCMS with two columns of different polarity to avoid coelutions. The most efficient setup has been to use two columns connected to two different split/splitless injectors mounted straight into the interface of one mass spectrometric detector.^[2] The scientific committee on consumer safety proposed to extend that list and it was documented (SCCS/1459/11). Here we report about 59 chemical substances using a twin-line (unpolar and WAX) GCMSMS method in order to apply maximum selectivity by selecting optimized multi reaction monitoring (MRM).

▪ Instrumentation

All analysis presented in this article were obtained with a Shimadzu GCMS-TQ8040 triple quadrupole GCMS quipped with an AOC-6000 auto-injector. Very good sensitivity combined with selectivity is achieved when MS/MS (MRM) mode is used. To separate all allergens a twin line setup was applied. From two injection units a Rxi-5SilMS and a Stabilwax (both from Restek, each with 15 m×0.15 mm×0.15 µm) were simultaneously mounted to the interface of the MS without connectors. Another advantage is the choice of column by the method and no change of configuration is necessary. The AOC-6000 can inject into either line.

▪ Experimental Conditions

1 µL of sample was injected using split mode with a split ratio of 1:100 at 240 °C for both lines. The GC oven program was set to 50 °C for 1 min, then increased by a rate of 20 °C/min to 120 °C, to 155 °C with a rate of 6 °C/min and up to 250 °C with 20 °C/min and hold for 2 min. Helium as carrier gas was selected with a linear velocity of 50 cm/sec constant over the run. With these settings the last Peaks elute below 16 min on the RTX5 and 17 min on the WAX, respectively. MS interface and ion source were set to 230 °C and 200 °C, respectively. As collision gas Argon was applied with a pressure of 200 kPa.

Table 1 shows the list of allergens with number (No.), retention times on both columns and MRM transitions. Due to product ion scans (PIS) and MRM optimization different transitions were used at the other column for four allergens (ID 11, 15, 23, 54). For most compounds a second qualifier was monitored which is not shown in Table 1. In addition, two internal standards were used. Up to a retention time of 8.5 min all targets were referred to 1,4-dibromobenzene, all other to 4,4'-dibromobiphenyl. Perfume samples were quantified by an internal four-point calibration on both columns, respectively. The results were checked in comparison to the supplier data and the results obtained by GCMS SQ in single ion monitoring (SIM) mode on two different columns.

#	Compound		Ret. Time 5SiIMS [min]	Ret. Time WAX [min]	Quantifier	CE	Qualifier I	CE
1	alpha-Pinene	Target	2.95	1.27	136.20>93.10	11	121.10>93.10	7
2	Benzaldehyde	Target	3.20	4.63	106.10>77.10	17	106.10>51.10	27
3	beta-Pinene	Target	3.35	1.74	136.10>93.10	11	121.20>93.10	7
4	alpha-Terpinene	Target	3.69	2.22	121.10>93.10	7	136.20>121.20	11
5	Limonene	Target	3.79	2.36	136.20>94.10	5	121.10>93.10	7
6	Benzyl alcohol	Target	3.83	7.90	108.10>79.10	19	108.10>90.10	9
7	Salicylaldehyd	Target	3.92	5.83	121.10>65.10	15	121.10>93.10	11
8	alpha-Terpinolene	Target	4.28	2.96	136.20>121.10	11	121.10>93.10	7
9	Linalool	Target	4.37	4.78	121.20>93.10	9	121.20>77.10	23
10	Camphor	Target	4.81	4.55	152.20>108.10	5	152.20>81.20	15
11	beta-Terpineol	Target	4.97		93.10>77.10	15	121.20>93.00	9
12	Menthol	Target	5.06	5.40	121.10>93.10	9	136.20>121.10	9
13	1,4-dibromobenzene	ISTD	5.22	5.47	123.20>81.10	9	138.20>95.10	9
14	Methyl-2-octynoate	Target	5.22	6.22	235.80>154.90	19	235.80>157.00	21
15	alpha-Terpineol	Target	5.20	5.57	123.10>67.10	7	123.10>53.10	13
16	gamma-Terpineol	Target	5.27	5.20	93.10>77.10	15	121.20>93.00	9
17	Methyl salicylate	Target	5.20	6.01	120.10>92.10	11	152.10>120.00	7
18	Citronellol	Target	5.50	6.75	123.20>81.10	9	138.20>95.10	9
19	Neral	Target	5.63	6.68	94.10>79.10	11	109.10>67.00	9
20	Linalyl acetate	Target	5.73	5.85	136.15>93.10	11	136.15>121.10	7
21	Geraniol	Target	5.74	4.85	123.20>81.10	11	123.20>79.10	19
22	Carvone	Target	5.71	7.58	82.10>54.10	5	108.20>93.10	9
23	Geranial	Target	5.94	6.31	152.20>137.20	5	137.20>95.10	11
24	Cinnamaldehyde	Target	6.02	6.32	94.10>79.10	11	109.10>67.00	9
25	trans-Anethole	Target	6.17	9.89	131.10>77.10	25	131.10>103.10	11
26	Hydroxy citronellal	Target	6.14	7.32	147.10>91.10	15	148.10>105.10	19
27	Anise alcohol	Target	6.12	8.76	96.10>81.10	7	121.20>93.10	7
28	DMBCA	Target	6.12	12.23	138.10>109.10	9	109.10>77.10	19
29	Cinnamyl alcohol	Target	6.50	6.72	132.10>117.10	15	132.10>115.10	25
30	Eugenol	Target	6.39	12.26	134.10>92.10	7	134.10>105.10	15
31	delta-Damascone	Target	6.96	11.38	164.10>149.10	9	164.10>131.10	11
32	Geranyl acetate	Target	7.23	6.63	192.20>135.10	13	123.20>81.10	7
33	beta-Damascenone	Target	7.27	6.58	136.10>121.10	11	121.10>93.10	11
34	alpha-Damascone	Target	7.33	7.23	121.10>105.10	11	121.10>77.10	23
35	Methyl isoeugenol	Target	7.47	6.90	192.20>135.10	9	123.20>81.10	11
36	beta-Damascone	Target	7.57	9.66	178.20>107.00	15	178.20>163.00	7
37	Vanillin	Target	7.73	6.41	192.20>177.10	11	177.20>121.10	19
38	Majantol	Target	7.52	13.81	152.10>123.10	19	152.10>81.10	23
39	Ebanol 1	Target	7.78	11.03	106.10>91.10	11	106.10>65.10	27
40	Caryophyllene	Target	8.19	7.96	121.10>93.10	9	121.10>77.00	21
41	Ebanol 2	Target	7.93	5.13	133.10>105.10	11	133.10>91.10	11
42	Isoeugenol	Target	8.32	8.40	164.20>149.20	7	121.20>77.10	9
43	Coumarin	Target	8.26	12.68	164.10>149.10	11	164.10>77.10	25
44	alpha-Isomethylionone	Target	8.12	13.20	146.10>118.10	13	118.10>89.10	21
45	Eugenyl acetat	Target	8.66	7.54	150.10>135.10	9	135.10>91.10	17
46	Lilial	Target	9.27	12.09	164.10>149.10	9	164.10>77.10	29
47	propylen phthalide	Target	9.47	9.97	189.20>131.10	11	189.20>91.10	21
48	Amyl salicylate	Target	10.14	13.07	159.10>103.10	15	159.10>77.10	27
49	Isoeugenol acetat	Target	10.23	10.68	138.10>120.00	7	208.10>138.00	5
50	Iso E Super 1	Target	10.71	13.11	164.10>149.10	9	164.10>77.10	27
51	Amyl cinnamaldehyde	Target	11.39	10.06	191.20>121.10	11	191.20>109.10	13
52	Iso E Super 2	Target	11.21	12.05	202.20>129.10	9	145.10>117.10	9
53	alpha- & beta-Santalol	Target	11.47	10.27	191.20>135.20	9	191.20>109.20	15
54	Lyrar	Target	11.56	12.65 / 13.11	94.10>79.10	11	93.10>77.00	15
55	Iso E Super 3	Target	11.43	13.37	136.10>79.10	11	136.10>108.10	3
56	Amyl cinnamyl alcohol	Target	11.73	10.86	107.10>79.10	7	177.20>149.20	5
57	Farnesol	Target	11.66	13.68	135.20>107.10	7	191.20>121.00	9
58	Hexyl cinnamaldehyde	Target	11.96	12.72	133.10>55.00	11	133.10>115.00	11
59	Acetyl cedrene	Target	12.22	12.72	136.20>121.10	9	121.10>93.10	7
60	Benzyl benzoate	Target	12.41	11.77	216.20>129.10	7	216.20>145.10	21
61	Galaxolide 1 & 2	Target	12.41	14.09	105.10>77.10	15	105.10>51.10	27
62	Benzyl salicylate	Target	12.41	14.09	194.10>165.10	25	212.10>105.10	7
63	Hexadecanolactone	Target	13.00	12.41 / 12.46	243.20>213.20	11	258.20>243.20	11
64	4,4'-Dibromobiphenyl	ISTD	13.19	14.78	228.10>91.10	15	228.10>210.10	5
65	Benzyl cinnamate	Target	13.60	12.67	83.10>55.10	7	83.10>68.10	15
66	Sclareol	Target	14.06	15.58	311.90>152.20	29	152.10>126.10	21
			14.56	16.57	192.10>189.10	29	238.10>193.10	7
			15.21	16.31	95.10>55.10	19	95.10>67.10	11

Table 1: Allergens with ID, retention times for Rxi-5SiIMS and Stabiwax column and MRM transitions.

Results

1. Calibration:

Calibrations for β - and γ -terpineol, Iso E super 1 and 3 and santalol were done in between 0.5 and 40 ppm ($\mu\text{g/mL}$), while all other allergens were calibrated between 2 and 100 ppm. The internal standard calibration curves as well as all samples were related using standard smoothing in GCMS Solution.

The calibration curves of a selection of allergens on the 5SiIMS column are shown in Figure 1. Regression coefficients were $R^2 > 0.999$ for all curves except of α -pinene (ID1, 0.998) and farnesol (ID57, 0.997) on the 5SiIMS column and β -damascone (ID36, 0.996) and hexadecanolactone (ID63, 0.995) on the WAX column.

2. Quantification of real samples:

Figure 2 shows the MRM traces of selected allergens for one of the perfume samples tested. The selectivity of these compounds in MRM mode provides good separation from matrix. Concentrations were calculated based on the internal standard calibration and are shown in Table 2 for that sample. The quantitative results were compared to the supplier data and the results obtained with Single Quad (twin-line/SIM mode) data.

Even with MSMS using one column phase the quantitative results indicate some differences of several allergens compared to supplier data. ID21 and 36 show better precision on the 5SiIMS column whereas Iso E Super 1 and 2 (ID50 and 52) should be quantified on the WAX column. ID44 on the 5SiIMS correlated with the SIM results and on the WAX column it fits to the supplier data indicating that here MRM on the Wax is more selective. For the compounds with ID9, 18 and 58 the results on both columns matches with the supplier data and the results obtained in SIM mode.

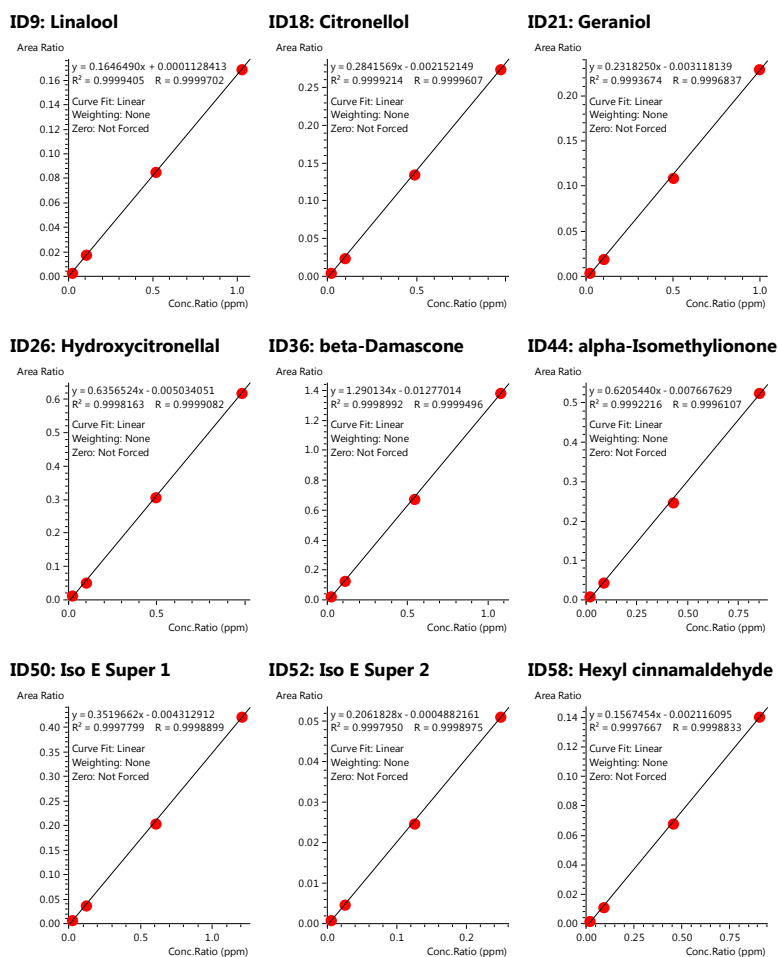


Figure 1: Calibration curves for selected allergens on 5SiIMS.

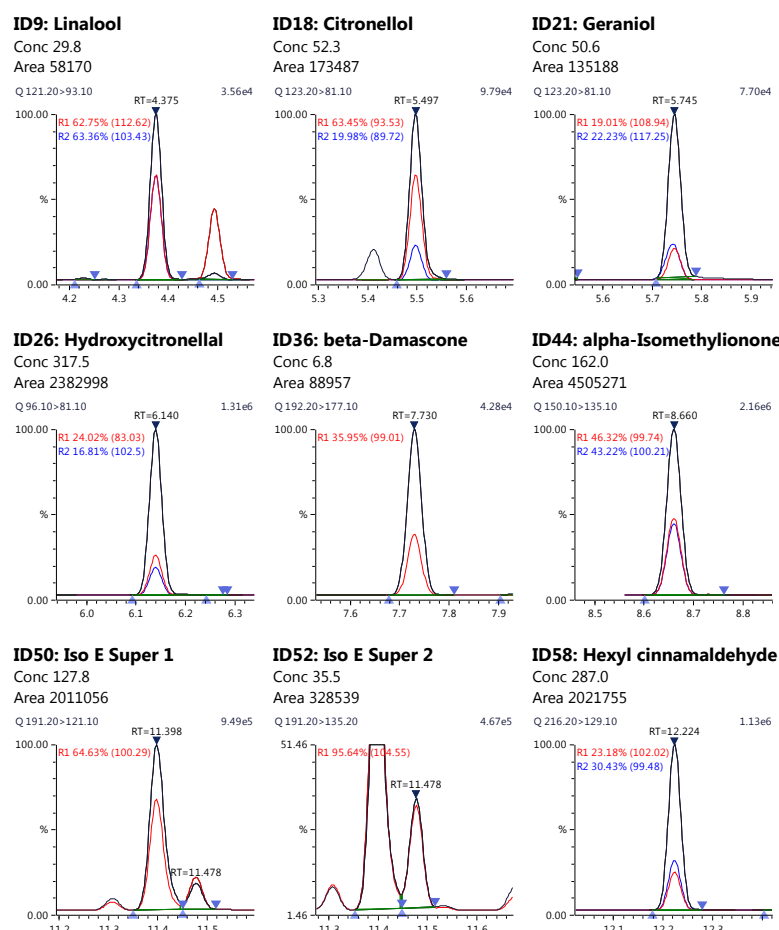


Figure 2: Selected allergens in a perfume sample on 5SiIMS.

ID	Compound	% according supplier	% twin-line SIM	% 5SiIMS MRM	Rel. to supplier	Rel. to twin-line SIM	% SH-WAX	Rel. to supplier	Rel. to twin-line SIM
9	Linalool	0.2701	0.2648	0.2683	-0.7	1.3	0.2713	0.4	2.5
18	Citronellol	0.4358	0.4164	0.4516	3.6	8.5	0.4443	2.0	6.7
21	Geraniol	0.4445	0.4459	0.4561	2.6	2.3	0.4808	8.2	7.8
26	hydroxycitronellal	3.1899	2.9807	2.9606	-7.2	-0.7	2.9870	-6.4	0.2
36	beta-Damascone	0.0631	0.0623	0.0615	-2.5	-1.3	0.0597	-5.4	-4.2
44	alpha-Isomethylionone	1.5458	1.4435	1.4599	-5.6	1.1	1.5451	0.0	7.0
50	Iso E Super 1	-	1.2304	1.1514	-	-6.4	1.2069	-	-1.9
52	Iso E Super 2	-	0.3414	0.3201	-	-6.2	0.3422	-	0.2
58	Hexyl cinnamaldehyde	2.5425	2.4492	2.5854	1.7	5.6	2.5832	1.6	5.5

Table 2: Supplier data and results of GCMS SQ (twin-line/SIM mode) compared to MRM on two columns for one perfume matrix.

Conclusion

Determination of 59 allergens were tested for different perfume matrices and can be performed with high sensitivity and selectivity using the GCMS-TQ8040 applying fast GCMSMS. Other matrices will be tested in a subsequent study. Regression coefficients of the calibrations were observed to be $R^2 > 0.999$ for many allergens.

As a conclusion it can be said that the determination of the extended list of potential allergens in perfumes were within 8% variation on both columns relative to the expected concentrations according to the supplier data. In general the comparison to SIM data indicate a better selectivity with MRM in comparison to SIM as expected. Twin-line setup with MS/MS provides the sufficient separation efficiency and selectivity to quantify allergens in perfume matrices.

The use of fast GCMSMS allows a drastic increase in sample throughput by obtaining high quantitative precision.

Acknowledgments

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References

- [1] EU 1223/2009.
- [2] H. Leijts et al: J. Agric. Food Chem. 2005, 53, 6487.



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