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Application News

No. SCA_280_091

Gas Chromatography Mass Spectrometry (GCMS)

Determination of 59 potential Allergens in Perfumes by Comprehensive GCXGC(qMS)

▪ 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 (unipolar and Wax) to avoid coelutions. The most efficient setup has been 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). To increase selectivity for the compounds in different matrices the addition of an analytical dimension can help avoiding false negative (or positive) identification. Recently we reported the use of a twin line GCMSMS setup.[3] Here we report about 59 chemical substances using comprehensive GCXGC(q)MS using a high speed acquisition quadrupole GCMS (50 scans/second over a mass range of 295 u at 20000 u/sec).

▪ Instrumentation

All analysis results presented in this article were obtained with a Shimadzu GCMS-QP2020 single quadrupole GCMS quipped with the patented advanced scanning speed protocol (ASSP, Patent US6610979). The travel time of the ions through the quadrupole towards the detector increases with increasing m/z which leeds to spectra skewing at high scanning speeds (>10000 u/sec). At high scanning speeds the ASSP automatically applies an accelerating voltage. This avoids skewing of the spectra observed with standard GCMS systems at such scanning rates. A ZOEX-1 two stage thermal modulator (ZOEX Corp., USA) was used and a AOC-20i auto-injector. In the first dimension a RXI-35MS 60 m, 0.25 mm, 0.25 µm column and in the second dimension a Wax 1.5 m, 0.15 mm 0.15 µm was used, respectively. The latter was placed into an additional GC-2010 Plus to control wrap around. The modulator was installed also in that GC. Data analysis was done using Chromsquare software, Shimadzu, for GCXGCMS data elaboration.

▪ Experimental Conditions

The modulation frequency was set to 5 seconds. 1 µL of sample was injected using split mode with a split ratio of 1:100 at 240 ˚C injector temperature. The GC oven program for the first (second) dimension was set to 60 ˚C (90 ˚C) for 0.5 min, then increased by a rate of 3 ˚C/min to 260 ˚C, 12 min (25 min). This offset for the second dimension turned out to be the optimum offset value in combination with 5 sec modulation time. Helium as carrier gas was selected with constant pressure mode at 120 kPa. MS interface and ion source were set to 230 ˚C and 200 ˚C, respectively. The scan range was set to 40-334 u with a rate of 50 scans/sec (20000 u/sec).
Two internal standards were used. Up to a total retention time of 35 min all targets were referred to 1,4-dibromobenzene, all other to 4,4'-dibromobiphenyl. Perfume samples were quantified by a four-point internal standard calibration i.g. 2, 10, 50 and 100 ppm in acetone. Four different perfume matrices were analysed and the quantitative results were checked in comparison to the perfume supplier data and also to twin line GCMSMS data.[3]

- **Results**

Figure 1 shows the contour plot of the 50 ppm standard. All compounds were separated. The compounds are listed in table 1 together with the total retention time in minutes (contour plot horizontal axis) and second dimensional retention time in seconds (contour plot vertical axis).

In figure 2 a and b zoomed areas of figure 1 are shown to visualize separation.

In these figures it can be seen that also blobs which elute close together are well separated.
That was achieved by replacing a RXI-5MS 30 m, 0.25 mm, 0.25 µm by a RXI-35MS 60 m, 0.25 mm, 0.25 µm.

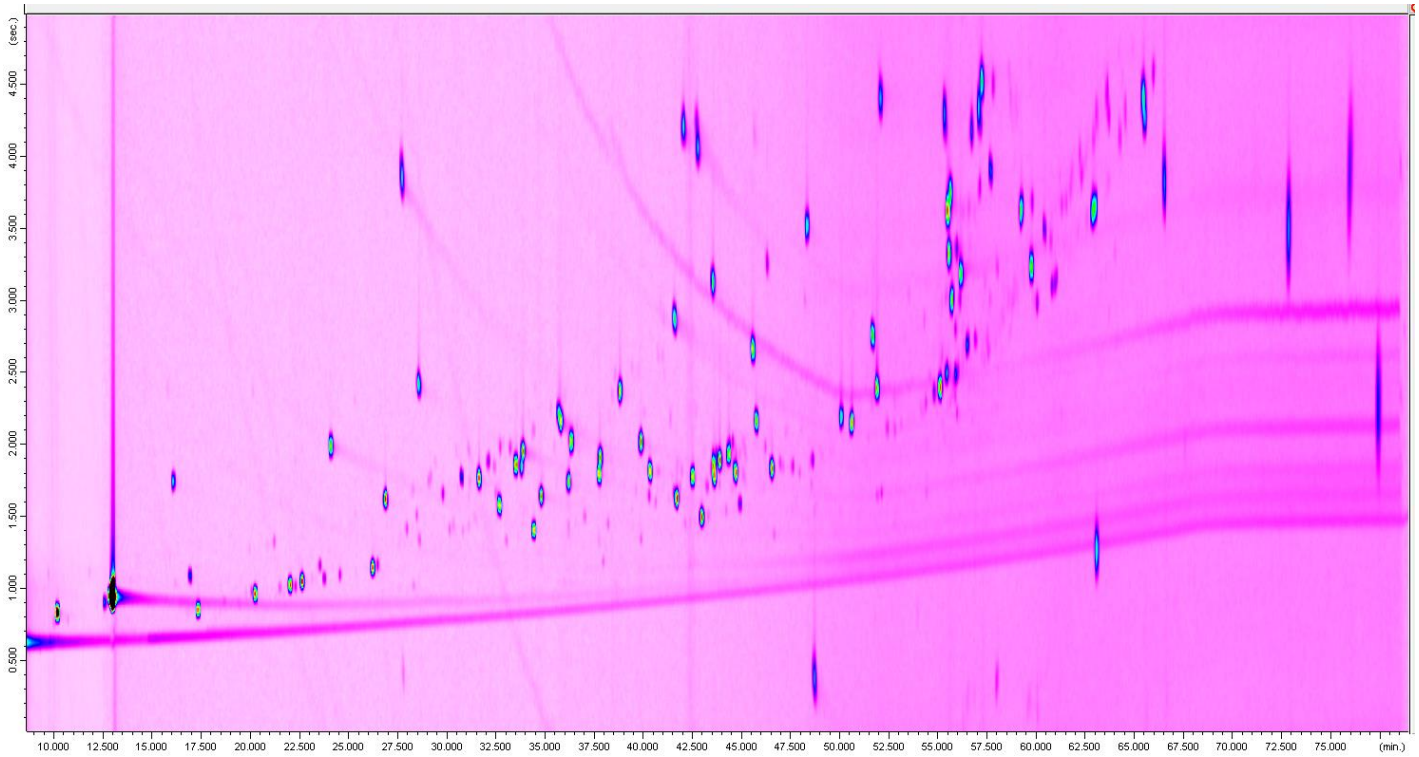

Fig 1: Contour plot of allergen standard at 50 ppm, Chromsquare comprehensive software.

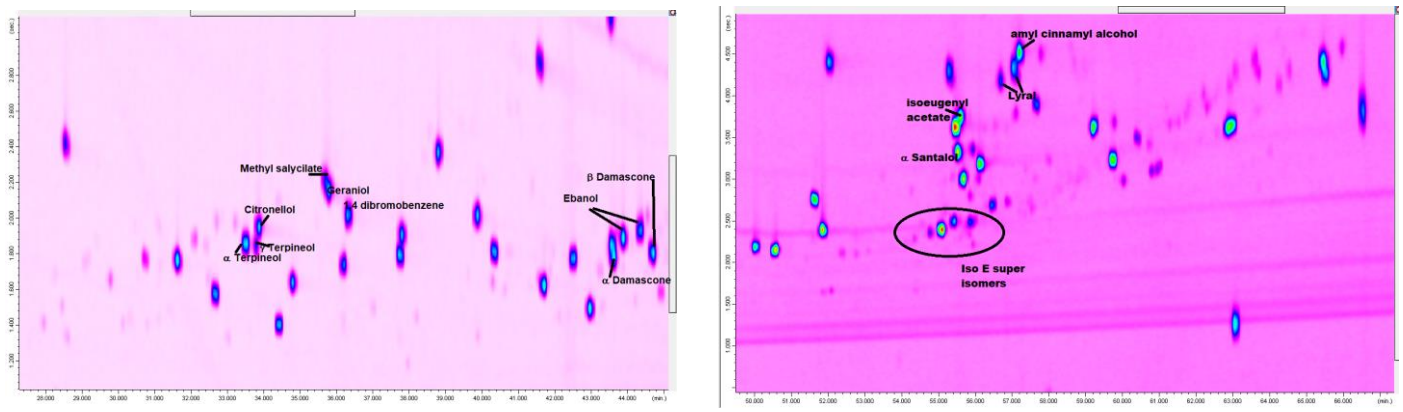

Fig 2: Zoomed section from fig 1, non labelled blobs see table 1.

Name	TtR (min)	2tR (sec)	Name	TtR (min)	2tR (sec)
alpha Pinene	17.30	0.86	Ebanol 2	44.40	1.94
beta Pinene	20.22	0.96	beta Damascone	44.73	1.80
alpha Terpinene	22.05	1.02	Majantol	45.58	2.68
Limonene	22.63	1.04	Methyleugenol	45.74	2.18
Benzaldehyde	24.07	2.00	alpha isomethyl Ionone	46.56	1.84
Terpinolene	26.22	1.14	trans-Isoeugenol	48.34	3.52
Linalool	26.89	1.62	Vanillin	48.71	0.38
Benzyl alcohol	27.76	3.86	Isoamyl salicylate	50.07	2.20
Salicylaldehyde	28.57	2.42	Lilial	50.65	2.16
Terpin-3-en-1-ol	29.81	1.66	Isoeugenyl acetate	51.66	2.78
beta trans Terpineol	30.73	1.78	amyl Salicylate	51.91	2.40
Menthol	31.65	1.78	Coumarin	52.11	4.42
Camphor	32.73	1.58	propiliden Phthalide	55.36	4.32
alpha Terpineol	33.56	1.86	Iso E Super 1	55.49	2.50
Citronellol	33.90	1.96	alpha Santalol	55.59	3.34
Linalyl acetate	34.47	1.40	alpha amyl Cinnamaldehyde	55.75	3.02
methyl Oct-2-ynoate	34.81	1.64	Iso E Super 2	55.91	2.50
methyl salicilate	35.57	2.42	Farnesol	56.17	3.20
Geraniol	35.82	2.16	Iso E Super 3	56.50	2.70
1 4 dibromoBenzene	36.32	2.02	Lyral	57.11	4.36
Geranial	37.73	1.80	Amyl cinnamyl alcohol alpha	57.28	4.54
Carvone	37.82	1.92	beta Santalol	57.77	3.90
hydroxy Citronellal	38.82	2.38	alpha hexyl Cinnamaldehyde	59.26	3.64
Anethole	39.90	2.02	Vertofix coeur	59.75	3.24
DMBCA	40.40	1.82	hexyl Cinnamaldehyde	59.85	3.70
Cinnamaldehyde	41.67	2.88	Galaxolide	62.93	3.62
Geranyl acetate	41.73	1.62	Galaxolide	63.01	3.66
Anisyl alcohol para	42.10	4.22	Benzyl benzoate	63.05	1.26
delta Damascone	42.56	1.78	Hexadecanolact-16-one	65.52	4.42
Caryophyllen	42.98	1,50	Hexadecanolact-16-one	65.61	4.28
Eugenol	43.59	3.14	Benzyl salicylate	66.60	3.82
alpha Damascone	43.65	1.78	4 4 dibromo Biphenyl	72.93	3.50
Ebanol 1	43.90	1.90	Benzyl cinnamate	76.01	3.86

Table 1: List of allergens with total retention time in minutes and 2 nd dimensional retention time in second.

Fig 3 shows the major modulated peak from the raw data for eugenol as an example. The width at the base is 300 msec and is representative for the other compounds. With 50 scans per second the difference between two scans was 20 msec. i.g. the number of data points across the modulated peaks are larger than 13 scans indicating enough data acquisition speed for very good quantitative analysis. However, the identification of the allergens need high spectrum quality. In the figure also the result of a point spectrum at peak rise, top and descend after background subtraction. The spectra are compared to the library (FFNSC 3, Shimadzu).

The search results shows similarity indices of SI= 92, 94 and 93, respectively. That proves firstly that the spectrum quality is not dependent on the concentration of the compound in the ion source and secondly the quality at the highest speed (20000 u/sec) is very similar to the library entries which where recorded with lower speeds (typically 3000 u/s). Even with single spectra at different peak positions similarity indices are higher than SI 92.

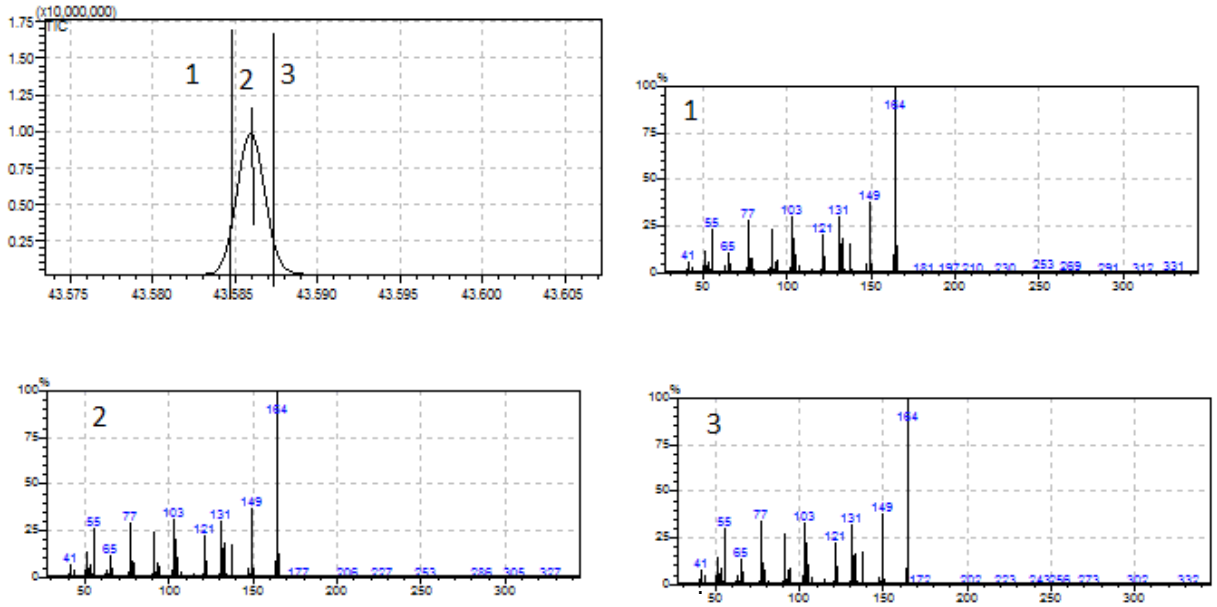

Figure 3: Major modulated Peak of eugenol and spectra at peak rise, top and descend after background subtraction. Similarity indices are SI = 92, 94 and 93, respectively (FFNSC 3 library).

▪ Quantification

In figure 4 calibration curves for various allergens are shown. The range of calibration was 2, 10, 50 and 100 ppm.

All correlation factors R^2 were larger than 0.9994.

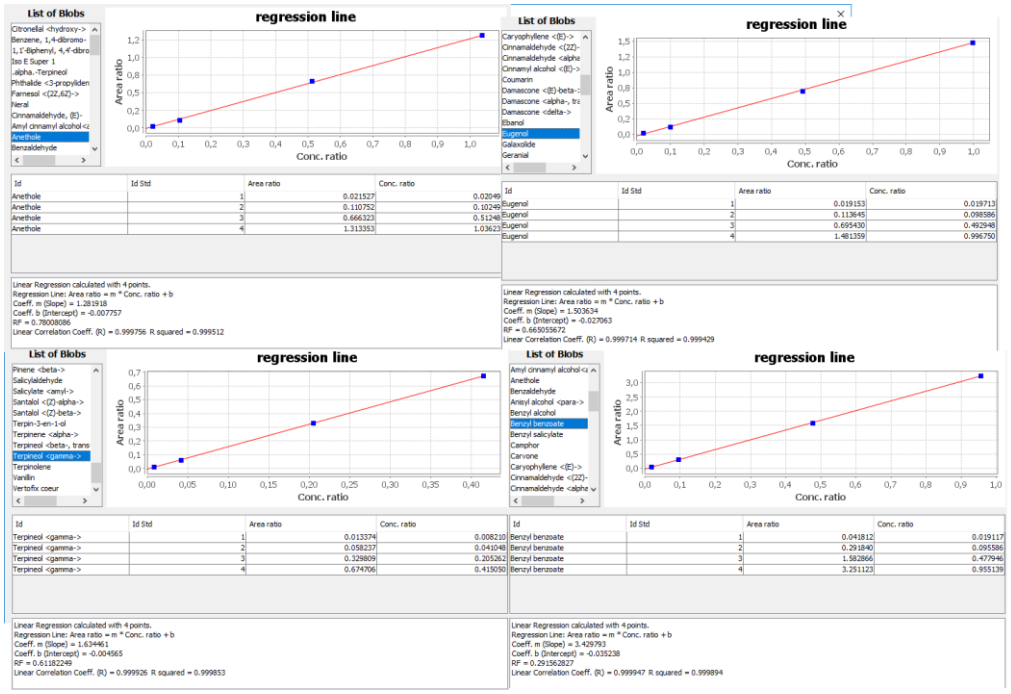

Figure 4: Selection of calibration curves calculated from the allergen blob areas.

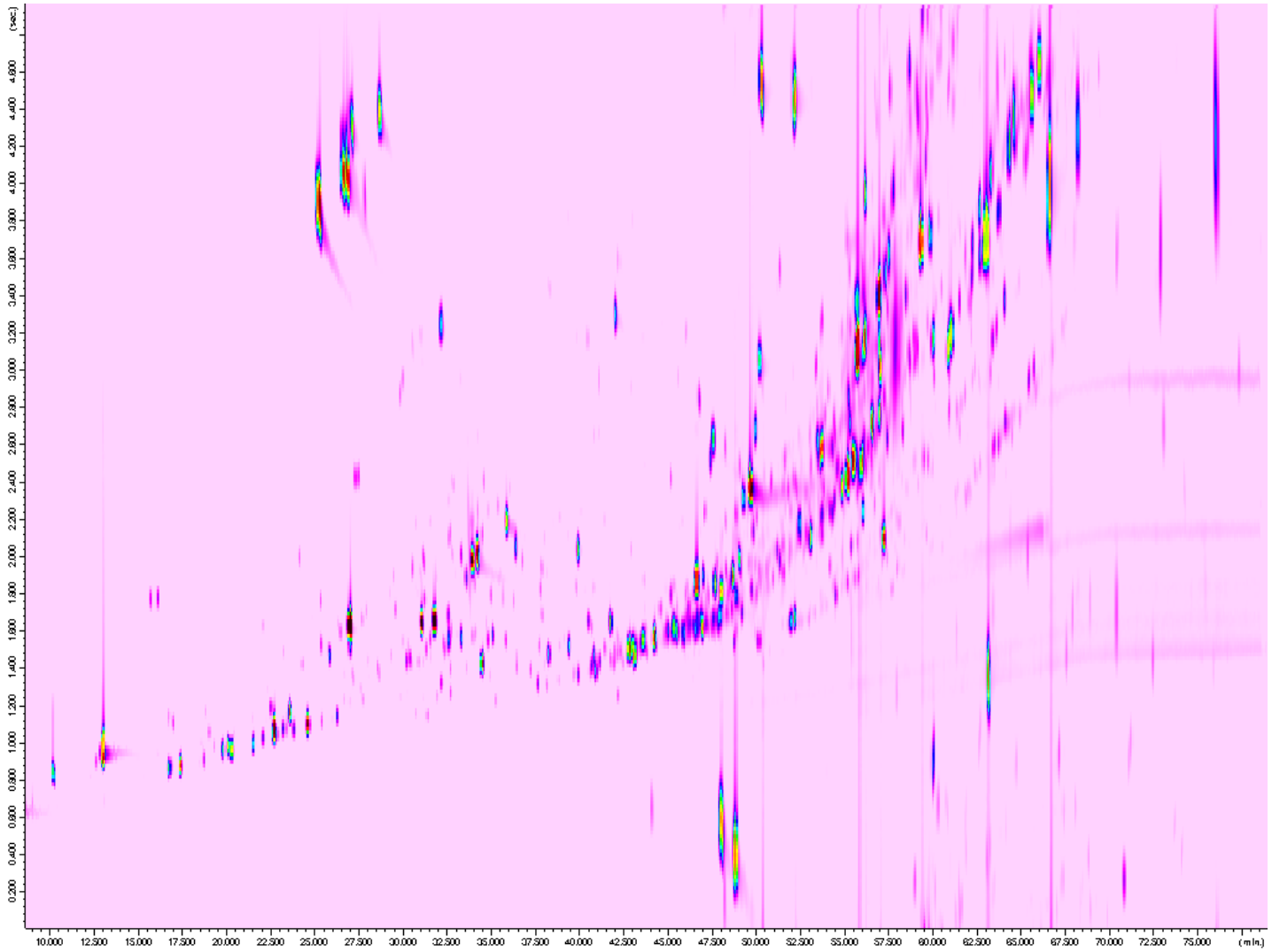

Figure 5: Contour plot of one of the perfume samples.

Fig 5 shows the contour plot of one of the perfume samples. In total 4 perfumes were tested with different dilutions 1:10, 1:100 and 1:1000, respectively, in acetone in order to quantify the allergens within the calibration range.
The quantitative results for the perfume shown in figure 5 are listed in table 2 in wt%.

The data obtained after quantification were compared to the perfume supplier data and also to the results obtained with twin line GCMSMS.[3]
The deviation between the concentrations obtained with the different method were below 7%.

Name	Ttr (min)	2nd (sec)	wt %	Name	Ttr (min)	2nd (sec)	wt %
alpha/beta Pinene	16.714	0.86	0.2610/0.2204	**Carvone**	37.816	1.96	0.0044
alpha Terpinene	22.051	1.02	0.0215	**Anethole**	40.067	2.04	0.1328
Limonene	22.635	1.10	6.2392	**Geranyl acetate**	40.644	1.62	0.1112
Benzaldehyde	24.150	2.00	0.0065	**alpha isomethyl Ionone**	45.583	2.98	3.0482
Terpinolene	26.219	1.14	0.0125	**Vanillin**	48.707	0.38	3.3888
Linalool	26.894	1.64	7.6065	**Coumarin**	52.192	4.48	1.8543
Benzyl alcohol	27.849	3.96	0.0207	**Iso E Super**	52.819	2.14	3.7765
alpha Terpineol	30.814	1.82	0.0190	**Iso E Super 1/2/3**	53.738	2.24	2.633/0.698/ 0.445
alpha-Terpineol	33.565	1.90	0.0207	**alpha isomethyl ionone**	56.564	1.88	3.0482
Linalyl acetate	34.474	1.44	0.3228	**Santalol**	57.264	3.84	0.1292
Citronellol	35.398	1.88	0.5779	**hexyl cinnam-aldehyde**	59.845	3.74	3.4942
Neral	36.229	1.76	0.0035	**Galaxolide**	62.930	3.78	6.0505
Geraniol	36.985	2.10	0.3004	**Benzyl benzoate**	63.306	1.34	1.1758
Geranial	37.814	1.84	0.0055	**Benzyl salicylate**	66.516	3.92	5.5703

Table 2: Results in wt% of one of the perfume samples tested.

▪ Conclusion

Determination of 59 allergens was performed for different perfume matrices with quantitative GCXGC(q)MS at high acquisition speed. The definition of high acquisition speed is not only based on scanning speed. A real fast acquisition needs more than one parameter i.g.

1. Number of scans per second to have more than 10 data point across each modulated peak of 300 msec at the base
2. The interscan delay must be small in order to achieve point 1
3. The mass range at such high acquisition frequency should be suitable for the application like in one dimensional GCMS
4. The spectra should not show any skewing relative to library spectra as this effects identification quality
5. The intensity at high 20000 u/sec should be comparable to low scanning speeds in order not to loose sensitivity drastically

In equivalence the scanning speed definition of 20000 u/sec is not enough to define a quadrupole to be a fast detector.

With standard GCMS systems usually a screwing of spectra are observed at high scanning speeds. The GCMS-QP2020 has a patented automatic acceleration of ions in the quadrupole to avoid increasing discrimination of ions with increasing m/z. This technique is called Advanced scanning speed protocol.[4]
Thus qualitative and quantitative analysis of the extended list of allergens with comprehensive GCXGC(qMS) can be done using the GCMS-QP2020.

▪ Acknowledgements

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▪ References

[1] EU 1223/2009.
[2] H. Leijs et al: J. Agric. Food Chem. 2005, 53, 6487.
[3] Shimadzu Application Note SCA_289_089.
[4] Shimadzu Application Note SCA_289_092.

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