POTENTIALS OF THE REVERSED-INJECT DIFFERENTIAL FLOW MODULATOR COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY IN THE QUANTITATIVE PROFILING OF SUSPECTED FRAGRANCE ALLERGENS

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Chiara Cordero[1], Davide Bressanello[1], Patrizia Rubiolo[1], Luigi Cobelli[2], Armando Miliazza[3], Matthew Giardina[4] and Carlo Bicchi[1]

1. Università degli Studi di Torino Turin - Italy
2. SRA Intruments SpA, Cernusco sul Naviglio, Milan, Italy
3. Agilent Technologies Italia SpA, Cernusco sul Naviglio, Milan, Italy
4. Agilent Technologies, Wilmington DE, USA

Aim and Scope

Multidimensional Gas Chromatography (MDGC) is a separation technique with great potentials in the (quantitative) profiling of very complex mixtures of volatiles [1] and, when implemented in "comprehensive" configurations (GC×GC), is capable of higher separation power, unmatched peak capacity and meaningful 2D elution patterns that facilitate analytes identification while providing distinctive sample's fingerprints.

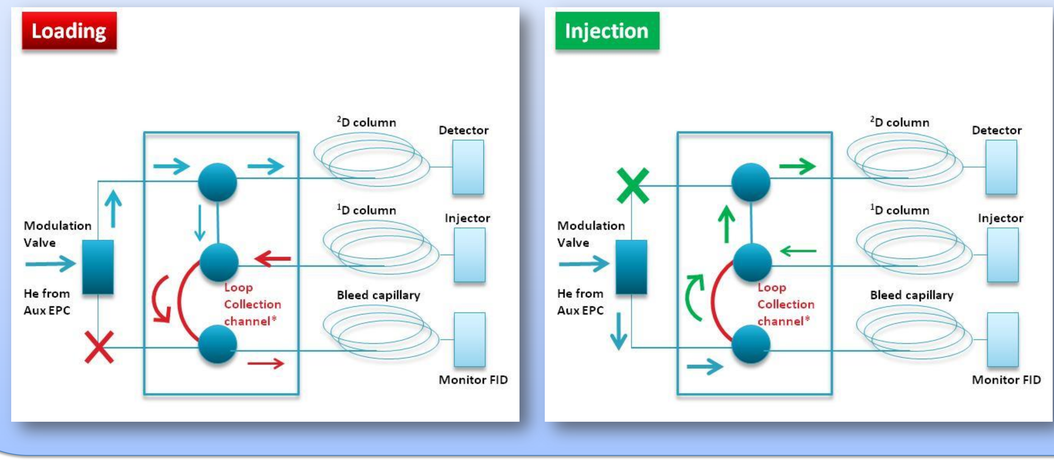
Thermal modulators, and in particular those implementing a cryogenic device [2], are widely used for volatiles' profiling because of their very efficient band focusing that avoids break-through phenomena. However, these modulators have also some drawbacks mainly related to the high costs in term of hardware and operations limiting their adoption for routine quality controls and high-informative throughput screenings [3].

In this study the first Capillary Flow Technology (CFT) reverse-inject differential flow modulator is implemented with different column configurations (lengths, diameters and stationary phase coupling) and detector combinations (Mass Spectrometry - MS and Flame Ionization Detection - FID) to evaluate its potential in the quantitative profiling of suspected allergens in medium-to-highly complex fragrances.

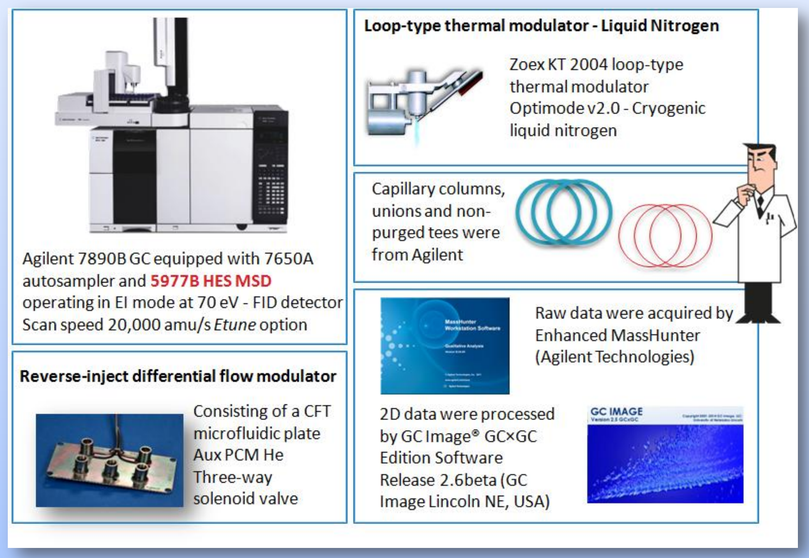
System performance parameters (^{2}D re-injection pulse width, 1D and 2D peak widths, retention times repeatability, MS sensitivity at high flow rates and MS fragmentation pattern reliability) are evaluated on a model mixture including 62 chemicals listed in the Scientific Committee on Consumer Safety (SCCS) Opinion on fragrance allergens in cosmetic products (1459/11 - 26/27 June, 2012). Within the different column combinations tested, the system demonstrating the best chromatographic performance has been selected for quantitative profiling of fragrance allergens.

References
[1] PJ Marriott et al. Trends Anal Chem 34 (2012) 1-21
[2] PQ Tranchida et al. Trends Anal. Chem. 30 (2011) 1347-1461
[3] C Cordero et al. J. Chromatogr. A 1147 (2015) 79-95

Reverse Inject Differential Flow modulated GC×GC



Experimental - GC×GC-MS/FID platforms



Samples
Pure standards of *n*-alkanes (from *n*-C9 to *n*-C25) for Linear Retention Indices (I^T_S) calibration were from Sigma-Aldrich (Milan, Italy).
Pure standards (or isomers mixtures) of volatile allergens, listed in **Table 1**, were purchased from Sigma-Aldrich (Milan, Italy) or kindly provided by Firmenich SA (Geneva, Switzerland).
Odorant mixtures and fragrances were prepared in author's laboratory by mixing exact quantities of pure raw materials and/or essential oils.

Results and Discussion

2D pattern reproducibility and quantitation accuracy

One of the main advantages of differential flow modulated GC×GC implemented with Capillary Flow Tecnology microfluidic plates concerns the reliability of modulation dynamics. **Table 2** reports experimental data on bi-dimensional retention times run-to-run reproducibility. Data were obtained with the "Alternative Configuration PEG-OV1701" analyzing a **20 mg/L** mixture **injected six times** in a three days time interval. Retention times (^{1}D and ^{2}D), Normalized 2D Peak Volumes (FID signal) and Normalized Percent Responses are reported as Means and Percent Relative Standard Deviation (RSD%).

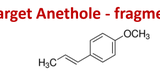
Table 2

	Retention I		Retention II		Volume Ratio		Percent Response	
	Mean	RSD %	Mean	RSD %	Mean	RSD %	Mean	RSD %
α-Pinene	2.58	1.06	0.91	0.61	1.01	5.29	2.32	5.07
ß-Pinene	3.11	1.61	1.02	1.57	0.16	10.25	2.22	7.33
Limonene	4.04	0.34	1.13	0.36	1.07	1.40	2.26	1.96
α-Terpinolene	5.11	0.27	1.25	0.21	0.36	0.57	0.82	0.69
Camphor	8.81	0.15	1.23	0.31	0.91	0.69	2.08	1.06
Benzaldehyde	8.83	0.00	0.73	0.83	0.34	1.50	0.78	0.94
Linalool	9.30	0.00	0.87	0.43	0.80	0.64	1.84	0.74
Linalyl acetate	9.57	0.00	1.33	0.34	0.70	1.41	1.61	1.78
trans-Caryophyllene	10.47	0.00	2.00	0.22	1.16	0.70	2.06	0.66
Menthol	10.97	0.12	0.93	0.59	0.86	0.50	1.97	0.46
Methyl Octinoate	11.13	0.00	1.00	0.38	0.67	0.36	1.53	0.74
Salicylaldehyde	11.53	0.00	0.70	0.37	0.39	1.01	0.91	1.13
Neral	11.70	0.00	1.13	0.23	0.72	0.41	1.65	0.55
α-Terpineol	11.97	0.00	0.88	0.36	1.26	0.54	1.89	0.61
ISTD 1	12.37	0.00	0.83	0.31	1.00	0.00	0.00	0.00
Carvone	12.60	0.00	1.02	4.84	0.17	11.84	1.11	12.68
Geranial	12.60	0.00	1.10	3.78	1.20	15.34	1.65	12.26
Geranyl acetate	13.10	0.00	1.21	0.35	0.74	0.68	1.71	0.86
ß-Citronellol	13.20	0.00	0.82	0.64	0.76	12.70	1.74	12.80
δ-Damascone	13.23	0.00	1.29	0.25	0.99	1.52	2.27	2.03
Methyl salicylate	13.26	0.13	0.81	0.39	0.39	16.32	0.72	19.61
DMBCA	13.27	0.00	1.05	0.26	0.71	1.34	1.63	1.17
α-Damascone	13.67	0.00	1.30	0.35	0.71	0.74	1.63	0.89
β-Damascone	14.08	0.12	1.32	0.29	1.22	2.13	2.81	2.67
ß-Damascenone	14.13	0.00	1.18	0.32	0.77	2.79	1.77	2.58
Anethole	14.17	0.00	0.87	0.36	1.11	1.98	1.56	1.45
Geraniol	14.53	0.00	0.75	0.34	1.00	0.83	2.31	1.03
α-Isomethylionone	14.67	0.00	1.48	0.17	0.75	0.64	1.73	0.74
Benzyl alcohol	14.90	0.00	0.55	0.50	0.67	0.73	1.53	0.91
Ebanol I	15.21	0.09	1.13	0.49	0.85	1.14	1.95	1.64
Ebanol II	15.77	0.00	1.07	0.19	0.79	4.13	1.81	4.52
Hydroxy citronellal	16.10	0.00	0.82	0.39	0.60	5.45	1.38	6.06
Cinnamaldehyde	17.53	0.00	0.73	0.72	0.39	2.22	0.91	2.76
Amyl Salicilate	17.64	0.08	1.11	0.74	0.37	13.62	1.59	13.21
Lilial	17.77	0.00	1.09	0.45	1.39	0.74	3.20	0.64
Iso E Super Isomer I	18.07	0.00	1.50	0.30	0.93	0.43	2.13	0.95
Majantol	19.00	0.00	0.74	0.27	1.05	1.05	2.42	0.84
Eugenol	19.50	0.00	0.66	0.57	0.63	0.69	1.44	0.66
Acetyl Cedrene I	20.60	0.00	1.42	0.18	0.83	2.83	1.90	3.40
Eugenyl acetate	20.86	0.08	0.79	1.22	0.61	2.28	1.21	3.83
Amyl cinnamic aldehyde	20.90	0.00	1.00	0.32	1.09	4.73	2.51	5.30
Anisyl alcohol	21.07	0.00	0.55	0.50	0.30	4.96	1.01	5.05
Cinnamyl alcohol	21.17	0.00	0.57	0.73	4.86	2.61	2.32	2.00
Acetyl Cedrene II	21.20	0.00	1.37	0.38	0.44	8.66	0.23	11.90
Musk G II	21.83	0.00	1.30	0.16	2.18	5.96	1.11	3.82
Musk G I	21.93	0.00	1.29	0.29	2.26	4.32	1.07	1.34
Farnesol Isomer I	21.97	0.00	0.89	0.50	1.97	5.69	0.97	2.43
Isoeugenol	22.07	0.00	0.64	0.66	1.63	11.24	1.38	4.95
α-Z-Santalol	22.21	0.08	0.87	0.73	3.43	6.91	1.08	0.94
Farnesol Isomer II	22.35	0.08	0.89	0.96	2.23	6.52	1.00	5.64
Hexyl cinnamic aldehyde	22.37	0.00	1.03	0.48	6.00	12.20	2.93	5.99
Hexadecanolactone	22.57	0.06	1.47	0.26	3.03	2.72	1.49	5.21
Iso Eugenyl acetate	23.17	0.00	0.77	0.53	3.40	6.86	1.67	1.06
β-Z-Santalol	23.33	0.00	0.86	0.37	2.13	3.66	0.80	3.91
Coumarin	23.40	0.00	0.68	0.30	2.14	10.80	1.05	4.44
Lyral major Isomer	23.73	0.00	0.82	0.25	1.74	5.57	2.00	1.54
Lyral minor Isomer	23.90	0.00	0.81	0.32	4.60	5.92	0.85	1.79
Amylcinnamic alcohol	24.67	0.06	0.72	0.95	2.08	5.78	1.02	3.74
Vanillin	24.90	0.00	0.56	0.46	2.41	5.84	1.18	1.45
6-Methyl coumarin	25.07	0.00	0.71	0.36	2.89	3.86	1.14	3.36
Benzyl benzoate	25.82	0.07	0.76	1.34	3.26	10.01	1.20	11.60
Benzyl salicylate	27.77	0.00	0.77	0.41	2.33	3.88	1.14	4.15
ISTD 2	29.97	0.00	0.80	0.73	1.00	0.00	0.00	0.00
Sclareol	31.73	0.00	1.00	0.26	2.42	2.12	1.19	7.65
Benzyl cinnamate	32.10	0.00	0.82	0.50	2.31	2.53	1.14	7.61

MS Reliability at high flow rates

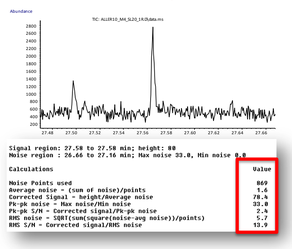
The differential flow modulated system coupled with the new **5977B Mass Spectrometer** implementing the **High Efficiency Source (HES)** provides:
1) stable acquisition frequency at the maximum scanning rate (20,000 amu/s);
2) highly reliable EI-spectra for confident analytes identification and confirmation;
3) improved sensitivity with up to 60 times higher S/N compared with previous generation instruments.

Target Anethole - fragment 148 m/z



Sample characteristics
Allergens mixture at 10 mg/L each in cyclohexane
Injection volume 1 µL;
S/SL injection; split ratio 1:20

MSD Model 5977A NO EMV GAIN
Outlet ^{2}D column flow ≈ 6 mL/min



MSD Model 5977B HES NO EMV GAIN
Outlet ^{2}D column flow ≈ 6 mL/min

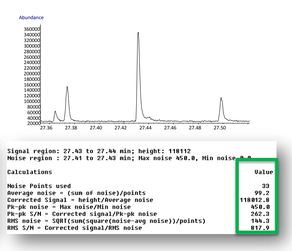
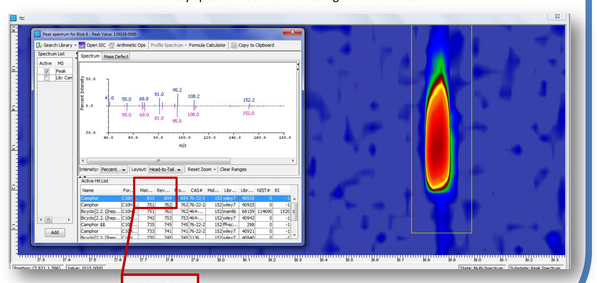


Figure 4

Camphor EI-MS spectrum - Identity confirmation
Allergens mixture at 2mg/L each in cyclohexane - Injection volume 1 µL;
S/SL injection; split ratio 1:20
2D Peak corresponding to Camphor - outlet ^{2}D column flow 6 mL/min
NIST Identity Spectrum Search - No background subtraction



Separation patterns and system performances

Table 1

Analytes		
α-Pinene	Methyl salicylate	Amyl cinnamic aldehyde
ß-Pinene	DMBCA	Anisyl alcohol
Limonene	α-Damascone	Cinnamyl alcohol
α-Terpinolene	β-Damascone	Musk G isomers
Camphor	Anethole	Farnesols Isomers
Benzaldehyde	ß-Damascenone	Isoeugenol
Linalool	Geraniol	Santalols
Linalyl acetate	α-Isomethylionone	Hexyl cinnamic aldehyde
trans -Caryophyllene	Benzyl alcohol	Hexadecanolactone
Menthol	Ebanol Isomers	Iso Eugenyl acetate
Methyl Octinoate	Hydroxy citronellal	Coumarin
Salicylaldehyde	Cinnamaldehyde	Lyral Isomers
Neral	Amyl Salicilate	Amylcinnamic alcohol
α-Terpineol	Lilial	Vanillin
Geranial	Iso E Super Isomers	6-Methyl coumarin
Carvone	Majantol	Benzyl benzoate
Geranyl acetate	Eugenol	Benzyl salicylate
ß-Citronellol	Acetyl Cedrene Isomers	Sclareol
δ-Damascone	Eugenyl acetate	Benzyl cinnamate

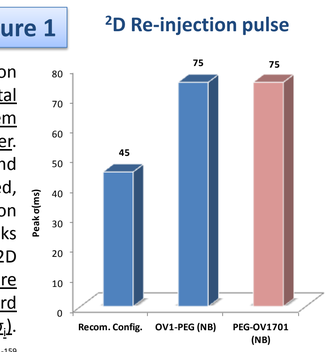
List of the analytes considered. They are part of the extended list of suspected allergens (Scientific Committee on Consumer Safety -SCCS; Opinion on fragrance allergens in cosmetic products 1459/11 - 26/27 June, 2012) that will be mandatorily monitored in cosmetic products.

Figure 1

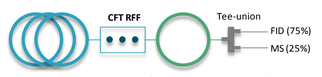
^{2}D Re-injection pulse

The ^{2}D re-injection efficiency is fundamental to maximize system separation power. Re-injection pulses band width is calculated, according Klee et al. [1] on un-retained solvent peaks in the middle of the 2D chromatogram. Values are reported as peak standard deviation ($^2\sigma_t$).

1. M. Klee et al. J. Chromatogr. A, 1383 (2015) 151-159



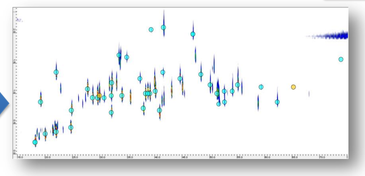
Agilent Recommended Configuration



^{1}D - Apolar SE52
30m×0.25mm×0.25µm
He carrier @ **0.35 mL/min**

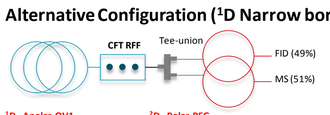
^{2}D - Medium polarity OV1701
5.0m×0.25mm×0.25µm
He carrier @ **25 mL/min**

Oven programming
80°C(2') to 280°C(10')@3°C/min
Modulation period: 2.5 s Injection: 0.11 s
Analysis time 75 min (last eluting *sclareol*)



Analysis time 75 min
Modulation period 2.5 s

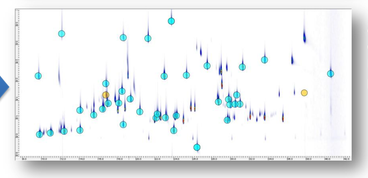
Alternative Configuration (^{1}D Narrow bore)



^{1}D - Apolar OV1
10m×0.10mm×0.40µm
He carrier @ **0.30 mL/min**

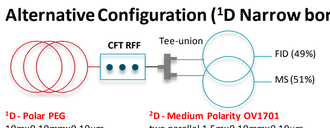
^{2}D - Polar PEG
two parallel 1.5m×0.10mm×0.10µm
He carrier @ 6 mL/min

Oven programming
70°C (1') to 280°C (10') @ 5°C/min
Modulation period: 4 s Injection: 0.20 s
Analysis time 40 min (last eluting *sclareol*)



Analysis time 40 min
Modulation period 4 s

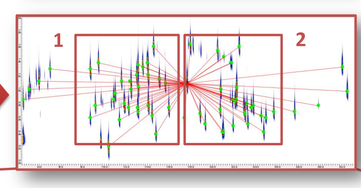
Alternative Configuration (^{1}D Narrow bore)



^{1}D - Polar PEG
10m×0.10mm×0.10µm
He carrier @ **0.30 mL/min**

^{2}D - Medium Polarity OV1701
two parallel 1.5m×0.10mm×0.10µm
He carrier @ 6 mL/min

Oven programming
70°C (0.48') to 260°C @ 6.2373°/min
Modulation period: 2 s Injection: 0.11 s
Analysis time 35 min
(last eluting *benzyl cinnamate*)



Analysis time 35 min
Modulation period 2 s

Figure 2

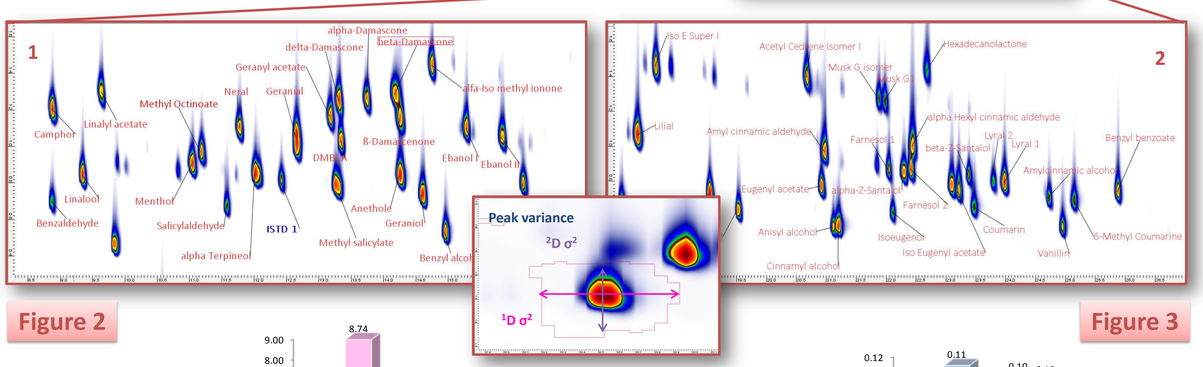
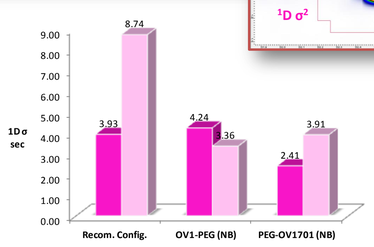


Figure 3

^{1}D peak-widths expressed as standard deviation ($^1\sigma_t$) and referred to the first eluting peak (α-pinene - pink bars) and last eluting peak (sclareol or benzyl cinnamate - light pink bars).



^{2}D peak-widths expressed as standard deviation ($^2\sigma_t$) and referred to the first eluting peak (α-pinene - dark blue bars) and last eluting peak (sclareol or benzyl cinnamate - light blue).

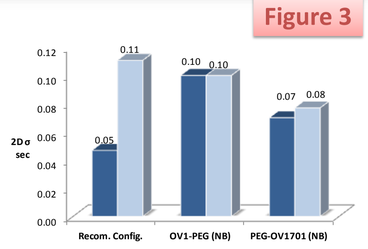
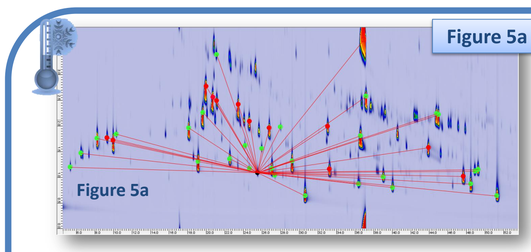
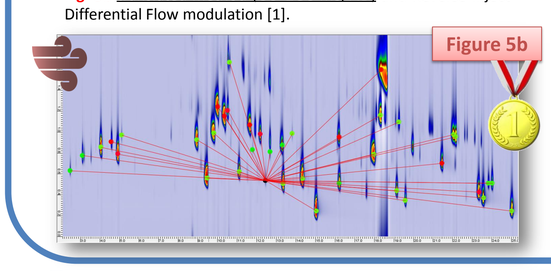


Figure 5a



Test mixture of potent odorants ranging from 15 to 0.05 g/L analyzed with:
Fig. 5a reference method GC×GC-MS/FID and loop-type thermal modulation (Zoex, KT2004 - Liquid N_2);
Fig. 5b translated method (GC×2GC-MS/FID) and Reverse Inject Differential Flow modulation [1].

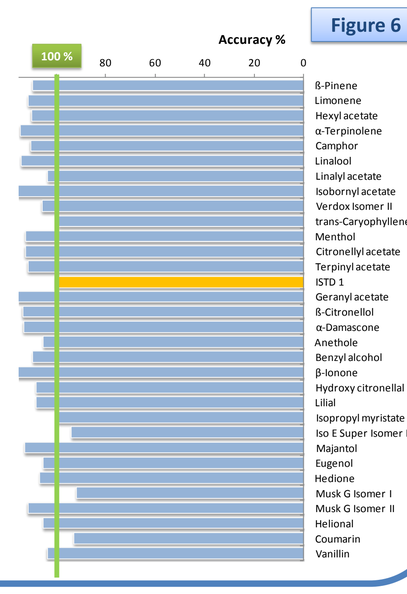
Figure 5b



The quantitation reliability (referred to as accuracy for simplicity) is evaluated taking as reference benchmark the estimated amount (g/L) derived from FID Response Factors.
GC×GC-MS/FID with thermal modulator is here arbitrarily considered as reference methodology to calculate the Quantitation Error % (or Bias %).

Quantitative profiling based on Response Factors, although unsuitable for conformity assessment (Commission Decision 657/2002) of fragrance samples, is widely adopted at the Quality Control level where large number of samples are subjected to pre-screening testing.
In this perspective GC×2GC-MS/FID with reverse inject differential flow modulation represent a valuable, highly effective platform.

Figure 6



Conclusions

GC×2GC with reverse inject differential flow modulation coupled with High Efficiency Source new generation quadrupoles represent a valuable tool for allergens screening in medium-to-high complexity fragrance materials. The system when configured with narrow bore ^{1}D columns provides high separation power, reliable identity confirmation, sensitivity up to sub-ppm level and confident quantitation based on Response Factors. All these characteristics make this platform suitable for high-throughput screenings of complex fragrance materials.

Acknowledgments

Firmenich