SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

Quantitative determination of 54 allergens in raw fragrances by comprehensive Two-Dimensional Gas Chromatography coupled to quadrupole Mass Spectrometry and Flame Ionization Detection (GC×GC-QMS/FID): a reliable approach for routine laboratories

Introduction

Reg. (EC) N°1223/2009 regulates the obligation to inform consumers of the presence of 24 fragrance substances identified as potential allergens in cosmetic products. In 2011 the Scientific Committee on Consumer Safety (SCCS/1459/11) extended the list of potential allergens to 57 fragrance substances including some isomeric forms or mixtures. In this context and in view of the forthcoming regulation on this extended list, the development of accurate and effective quantification methods is mandatory.
In November 2016 the Analytical Working Group of the International Fragrance Association (IFRA) published a document guideline illustrating the method procedure to "… identify and to quantify the volatile compounds which are suspected to be allergens in fragrance compositions and raw materials used in cosmetic products… the analytes covered are based on the contents of the SCCS Opinion document and as listed in the legislation proposed by the European Commission".

The analytical method is based on gas chromatography and mass spectrometry (GC-MS) and is capable to detect and to quantify about 60 fragrance substances and major isomers at a concentration higher than 0.002% (20 mg/kg) in ready to inject fragrance raw materials.
To match routine laboratory control requirements in term of rapidity and reliability of the results, comprehensive two-dimensional gas chromatography hyphenated with robust and low-cost quadrupole mass spectrometry (GC×GC-QMS) represent a strategic choice.

The present Application Note illustrates a method based on GC×2GC-QMS/FID aiming at accurately quantify 54 fragrance allergens within a range of concentrations of four orders of magnitude, i.e. 2-10,000 mg/kg in raw fragrance materials.

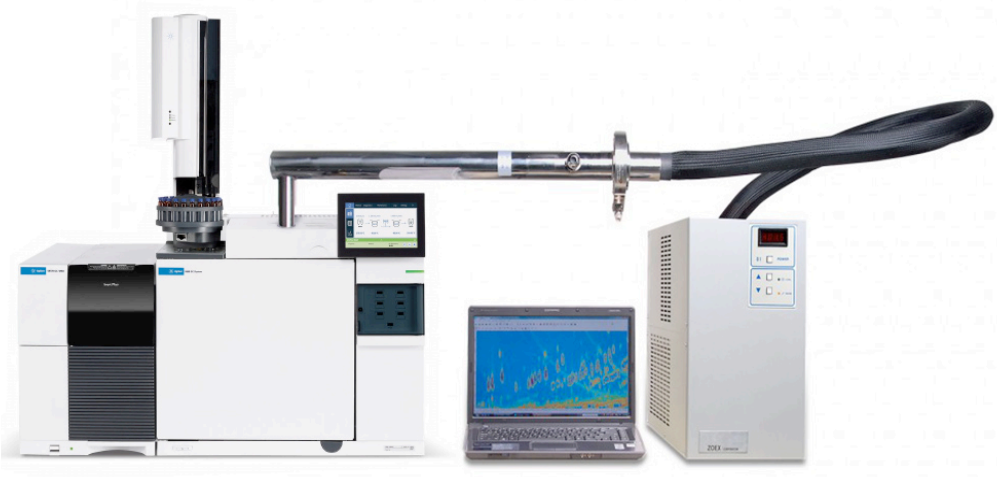


Fig. 1 – GCxGC-MSD/FID, Agilent Technologies

In the present study it was adopted a loop-type thermal modulator (KT 2004 Zoex Corporation, Houston, TX) with liquid nitrogen as cryogenic fluid; systems based on the same modulation dynamics but avoiding cryogenic fluids are equipped with chill units - ZX2 Zoex. **These solutions are fully compatible with allergens volatility for an effective trapping.**

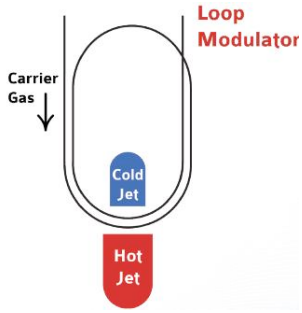
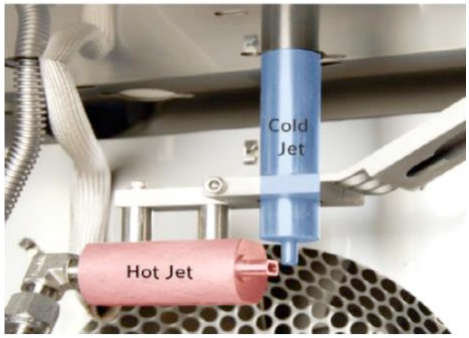


Fig. 2 – Loop-type thermal modulator - Zoex



The GC×GC technique with cryogenic modulators

The core of a GC×GC system is the modulator; the device accumulates, refocuses and rapidly releases fractions eluting from the first dimension (1D) into the second dimension (2D) column. These operations are run within a fixed time frame, named modulation period (PM), and repeated across the entire chromatographic run. Modulation has to be sufficiently rapid to preserve the original 1D separation while 2D separation has to be fast enough to be ended before the injection of the subsequent fraction from 1D effluent. Appropriate selection of columns dimensions, combination of stationary phases, PM and timing enables the full exploitation of system potentials, optimized peak capacity and high reproducibility in terms of 2D peaks distribution over the chromatographic plane and accurate mass transfer (accuracy in quantitative determinations). A schematic diagram of a GC×GC system is illustrated in Figure 3.

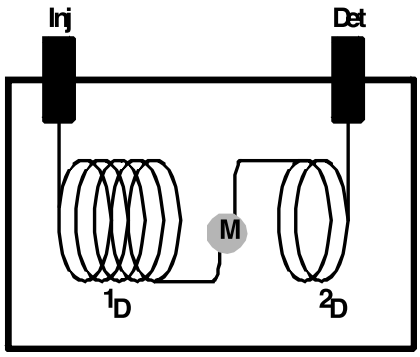


Fig. 3 – Schematic diagram of a comprehensive two-dimensional system. Inj: injector; Det: detector; M: modulator; 1D: first dimension column; 2D: second dimension column.

Parallel Detection MS/FID

The "third" system dimension is represented by MS detection; MS is mandatory for complex fragrance formulations, resulting in several co-elutions, and for macthing confirmatory requirements where the MS signature improves the consistency of quantitative data for regulated substances. The GC×GC hyphenation with rapid-scanning QMS brings great advantages in terms of system robustness and costs effectiveness for quality control laboratories.

The extension of the quantitation range over four orders of magnitude (2-10,000 mg/kg) is by parallel FID detection.
The list of allergens quantified is shown in Table 1 together with Target ions and Qualifiers adopted for confirmatory purposes.

Analyte - Chemical name	CAS # number	Purity (FID)	Ti	Q1	Q2
Amylcinnamic alcohol alpha	101-85-9	>99%	133	91	204
Anethole trans	4180-23-8	>99%	148	147	117
Anise alcohol	105-13-5	>99%	138	109	121
Benzyl alcohol	100-51-6	100%	79	108	107
Caryophyllene beta	87-44-5	99%	91	133	204
Cinnamyl alcohol	104-54-1	98%	92	134	115
Citronellol	106-22-9	>99%	69	41	156
Ebanol® E	1067999-31-8	45%	149	83	93
Ebanol® Z	1237530-53-8	45%	149	69	55
Eugenol	97-53-0	100%	164	149	131
Farnesol (E, E)	106-28-5	99%	69	81	93
Geraniol	106-24-1	99%	69	138	123
Isoeugenol E	5932-68-3	>99%	164	149	103
Isoeugenol Z [e]	5912-86-7	<1%	164	149	103
Limonene	138-86-3	>99%	68	67	136
Linalool	78-70-6	>99%	71	93	121
Menthol	89-78-1	>99%	81	71	95
Pinene alpha	80-56-8	>99%	93	91	136
Pinene beta	127-91-3	99%	93	79	69
Santalol alpha	115-71-9	52%	93	202	107
Santalol beta	77-42-9	23%	94	122	79
Sclareol	515-03-7	99%	69	191	177
Terpinene alpha	99-86-5	90%	121	93	136
Terpineol alpha	98-55-5	92%	136	121	93
Trimethylbenzene propanol (Majantol®)	103694-68-4	>9%	106	178	91
Propylidene phthalide-3 (E)	56014-72-3	95%	159	174	104
Propylidene phthalide-3 (Z) [e]	94704-89-9	4%	159	174	104
Acetylcedrene (Vertofix®)	32388-55-9	73%	161	147	119
Isoeugenyl acetate	93-29-8	>99%	164	149	206
Amylcinnamaldehyde alpha (Flosal®) (E)	122-40-7	93%	202	129	115
Amyl salicylate	2050-08-0	100%	120	138	208

Analyte - Chemical name	CAS # number	Purity (FID)	Ti	Q1	Q2
Benzaldehyde	100-52-7	100%	105	106	77
Benzyl benzoate	120-51-4	>99%	105	212	91
Benzyl cinnamate	103-41-3	99% [c]	131	192	91
Benzyl salicylate	118-58-1	100%	91	228	65
Butylphenyl methylproprional (Lilial)	80-54-6	98%	189	204	147
Camphor	76-22-2 / 464-49-3	99% [c]	95	152	108
Carvone	99-49-0 / 6485-40-1 / 2244-16-8	>99%	82	150	93
Cinnamaldehyde	122-40-7	96%	131	132	103
Neral = Citral (Z)	106-26-3	49%	69	41	134
Geranial = Citral E	5392-40-5	50%	69	152	84
Coumarin	91-64-5	100%	146	118	89
Damascenone beta (rose ketone-4)	23696-85-7	>99%	177	192	107
Damascone alpha	024720-09-0	97%	192	123	69
Damascone beta E	23726-91-2	96%	69	121	190
Damascone delta (rose-ketone-3)	57378-68-4	94%	192	123	69
Dimethylbenzylcarbinyl acetate (DMBCA acetate)	151-05-3	>99%	132	117	91
Eugenyl acetate	93-28-7	98%	164	206	149
Hexamethylindanopyran (Galaxolide® 1) [d]	1222-05-5	44%	213	228	128
Hexamethylindanopyran (Galaxolide® 2) [d]	1222-05-6	44%	213	228	128
Geranyl acetate	105-87-3	>99%	69	136	121
Hexadecanolactone / Dihydroambrettolide	109-29-5	99%	55	236	41
Hexylcinnamaldehyde alpha (Jasmonal®)	101-86-0	>99%	216	129	117
Hydroxycitronellal	107-75-5	96%	59	71	95
Lyral (minor) [e]	51414-25-6	26%	136	93	59
Lyral (major)	31906-04-4	73%	136	93	59
Isomethylionone alpha	127-51-5	88%	135	107	150
Linalyl acetate	115-95-7	98%	93	136	121
Methyl salicylate	119-36-8	100%	120	92	152
Folione	000111-12-6	>99%	95	123	79
Salicylaldehyde	90-02-8	100%	122	121	65
Terpinolene	586-62-9	95%	93	121	136
ISO E® alpha	68155-66-8	63%	191	119	43
ISO E® beta [e]	54464-57-2	31%	191	119	43
ISO E® gamma [e]	68155-67-9	5%	191	119	43
Vanillin	121-33-5	100%	151	81	152
1,4-dibromobenzene (ISTD1)	106-37-6	97%	236	238	234
4,4'-dibromobiphenyl (ISTD2)	92-86-4	97%	310	152	76

The 2D plot of the allergens standard mixture at 10 mg/L acquired by QMS is shown in Figure 4. Black circles locate ISTDs peaks while green circles correspond to allergens.

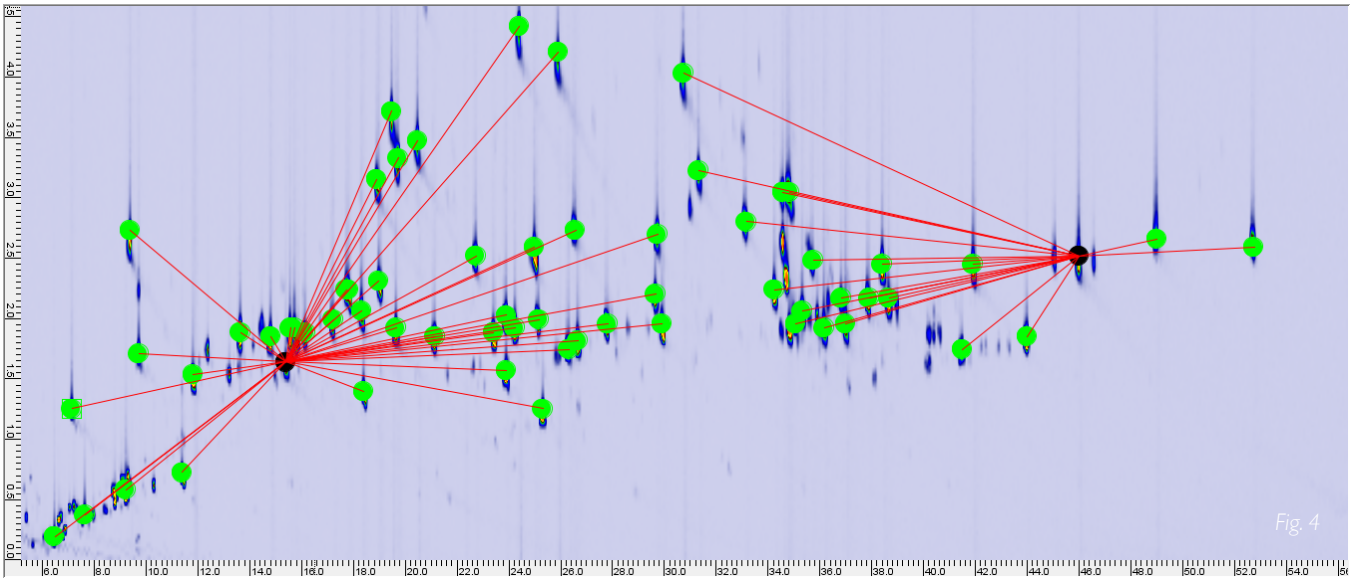


Fig. 4

Method figures of merit include a good repeatability and intermediate precision (Coefficient of Variation - CV%) on 2D peaks quantitative descriptors (Normalized Peak Volumes) and good accuracy [ref. Belhassen et al. Flavour Fragr J. 2018;33:63–74). Figure 5 shows CV % on 2D Peak Volumes from FID detection at 1 and 25 mg/L. Acceptability criteria were fixed in agreement with Commission Decision 657/2002.

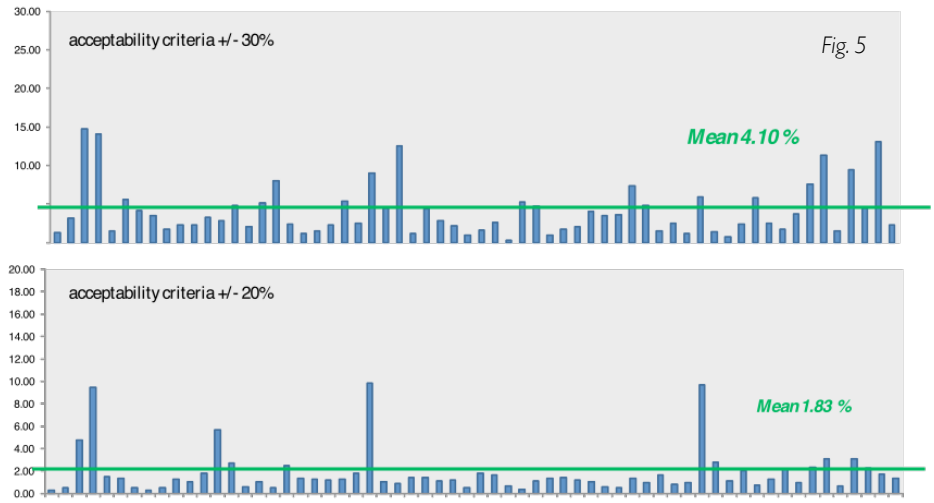
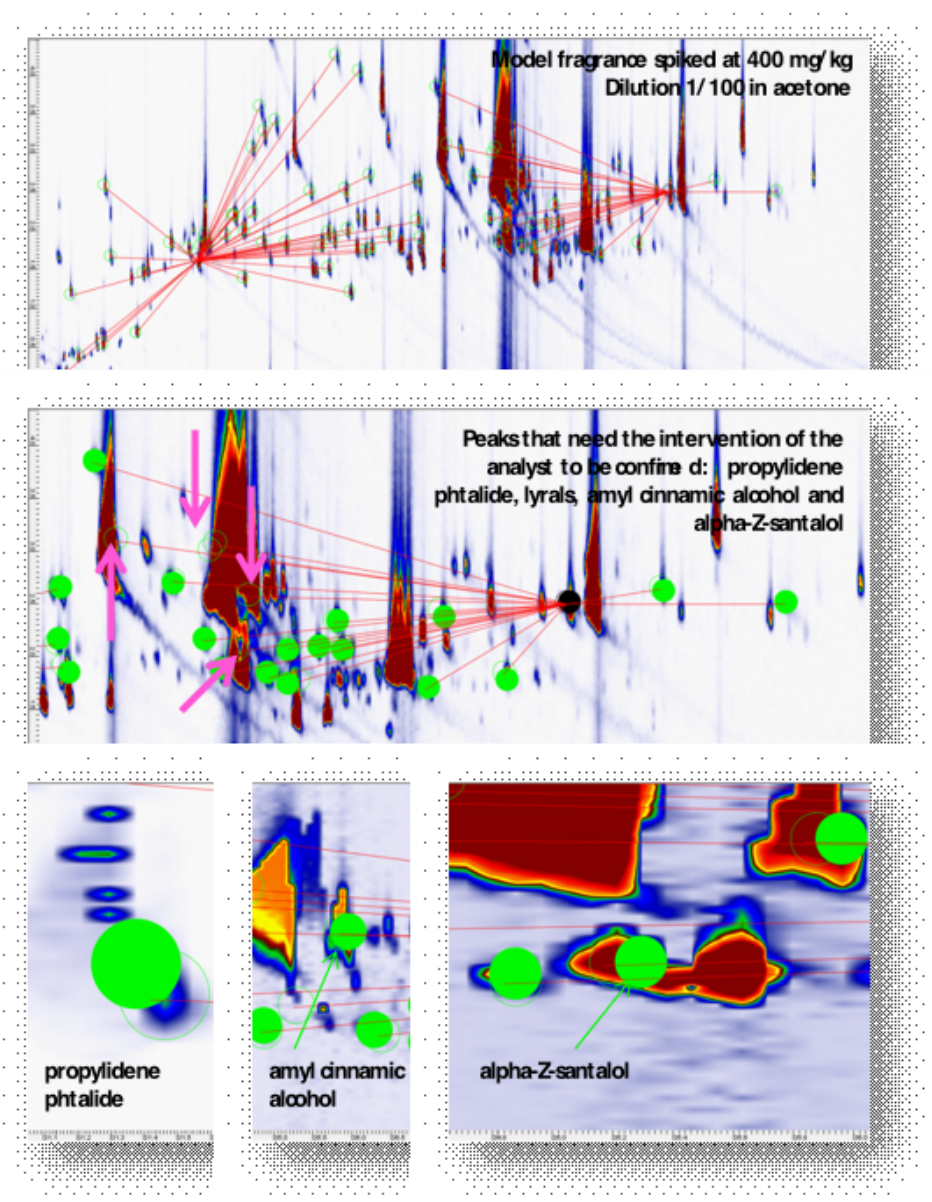


Fig. 5

Complex fragrances pose severe challenges on both analytes identity confirmation and quantitation; co-elution issues can be easily overcome by exploiting in full all the system informative dimensions as illustrated in Figure 6 where some analytes (propylidene phtalide, lyrals, cinnamic alcohol, amyl alcohol and alpha-Z-santalol) are masked by co-alution with fragrance major compounds. In these cases, GC Image software tools enable scripting and "isolation" of analytes response by specific ions response.



Acknowledgments:
Prof. Chiara Emilia Cordero and Prof. Carlo Bicchi at the Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin contributed to the development and validation of the Application Note. The work was done in collaboration with Firmenich SA (Geneva) and is the object of a publication: Belhassen et al Flavour Fragr J. 2018;33:63–74
For experiment details and publication reprints please directly contact the corresponding author:
chiara.cordero@unito.it

SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

SRA Instruments S.p.A
20063 Cernusco S/N (MI)
Tel +39 02 9214 3258
www.srainstruments.com
info@srainstruments.com

SRA Instruments SAS
69280 Marcy l'Etoile Lyon
Tel +33 04 7844 2947
www.srainstruments.com
info@sra-instruments.com