Comprehensive two-dimensional Gas Chromatography with conventional Inner Diameter Columns: method development and flow regime optimization

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Aims and scope

The configuration and optimisation of a GCxGC system require a more complex approach than that used for conventional 1D-GC; the separation in both dimensions is differently and independently influenced by temperature and carrier gas flow [1, 2, 3] and also by modulation period and temperature. In GCxGC the columns of a set are coupled in series therefore the carrier gas flow and velocity differently affect the separation of each column and, similarly to 1D-GC, the efficiency in both dimensions depends on the linear average velocity (u) of the carrier gas. Column flow optimization is a critical step for a GCxGC separation since changes in carrier gas velocity are expected to affect analyte resolution and elution order.
The present study aims to evaluate through the analysis of four test samples (i.e. n-alkanes, hydrocarbons, suspected allergens and fatty acid methyl esters), (i) the experimental possibility to apply a correct flow regime in both GCxGC dimensions by combining columns with a conventional ID, and (ii) to measure its effect on basic chromatographic parameters (i.e. theoretical Peak Capacity (*n*) and mono-dimensional and bi-dimensional Separation Measure (S_1, S_2 and S_{GCxGC})) and the critical GCxGC parameters such as degree of orthogonality (i.e. Separation Space Used).

[1] J. Dalluge, R.J.J. Vreuls, J. Beens, U.A.Th. Brinkman, J. Sep. Sci. 25 (2002) 201.
[2] R. Ong, P. Marriott, P. Morrison, P. Haglund, J. Chromatogr. A 962 (2002) 135.
[3] J. Beens, H.G. Janssen, M. Adahchour, U.A.Th. Brinkman, J. Chromatogr. A 1086 (2005) 141.

Experimental

Samples

Pure standard samples of n-alkanes (from C9 to C25), limonene, phenylacetaldehyde, linalool, benzyl alcohol, estragole, methyl 2-octynoate, citronellol, geraniol, citral, cinnamic aldehyde, hydroxycitronellal, anisyl alcohol, cinnamic alcohol, eugenol, methyl eugenol, α-isomethylionone, isoeugenol, butylphenyl methylpropional (lilial), coumarine, amyl cinnamic aldehyde, farnesol, amyl cinnamic alcohol, hydroxyisohexyl-3-cyclohexene carboxaldehyde (lyral), hexyl cinnamic aldehyde, benzyl benzoate, benzyl salicylate, benzyl cinnammate, and 1,4-dibromobenzene (ISTD-1). 4,4'-dibromodiphenyl (ISTD-2) were supplied by Sigma-Aldrich (Milan, Italy).
Solvents (cyclohexane, n-hexane, acetone) were all HPLC-grade from Riedel-de Haen (Seelze, Germany). Standard stock solutions were stored at – 18°C and used to prepare standard working solutions at suitable concentrations and stored at – 18°C.
The **Fatty Acids Methyl Esters mixture** was purchased from Supelco (Milan, Italy) and consisted of cis-13,16-docosadienoic acid methyl ester, cis-4,7,10,13,16,19-docosahexaenoic acid methyl ester, cis-11,14-eicosadienoic acid methyl ester, cis-5,8,11,14,17-eicosapentaenoic acid methyl ester, cis-8,11,14-eicosatrienoic acid methyl ester, cis-11,14,17-eicosatrienoic acid methyl ester, cis-11-eicosenoic acid methyl ester, methyl cis-10-heptadecenoate, methyl hexanoate, methyl γ-linolenate, methyl arachidate, methyl arachidonate, methyl behenate, methyl butyrate, methyl decanoate, methyl dodecanoate, methyl elaidate, methyl erucate, methyl heneicosanoate, methyl heptadecanoate, methyl linoleate, methyl linolelaidate, methyl linolenate, methyl myristate, methyl myristoleate, methyl oleate, methyl octanoate, methyl palmitate, methyl palmitoleate, methyl pentadecanoate, methyl cis-10-pentadecenoate, methyl stearate, methyl tricosanoate, methyl tetracosanoate, methyl tridecanoate, methyl undecanoate, methyl cis-15-tetracosenoate. Components range from a minimum of 2% to a maximum of 4% w/w.

The hydrocarbons **"Quantitative Reference Standard 512"** mixture (Boiling Point range 36-254°C) was purchased from AC Analytical Controls (Rotterdam, The Netherlands) and consisted of: cyclopentane, n-pentane, cyclohexane, 2,3-dimethylbutane, n-hexane, 1-hexene, methylcyclohexane, 4-methyl-1-hexene, n-heptane, 1,2-dimethylcyclohexane, 2,2,4-trimethylpentane, n-octane, 1,2,4-trimethylcyclohexane, n-nonane, n-decane, n-undecane, n-dodecane, benzene, toluene, trans-decalin, n-tetradecane, ethylbenzene, o-xylene, n-propylbenzene, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, 1,2,4,5-tetramethylbenzene, pentamethylbenzene. Components range from a minimum of 1.1% to a maximum of 5% w/w.

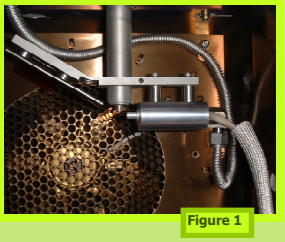

Figure 1

Instrumental set-up

Comprehensive GCxGC/qMS analyses were carried out on a Agilent 6890 GC coupled with a 5975 MS detector (Agilent, Little Falls, DE, USA) operating in electron impact mode at 70 eV. Ion source temperature: 230 °C, Quadrupole temperature 150°C, Transfer line: 280 °C. An automatic tuning was used. Scan range was from 35 m/z to 300 m/z and scan rate was set at 10000 amu/s. The system was provided with a two-stage thermal modulator, **Figure 1** (KT 2004 loop modulator from Zoex Corporation, Lincoln, NE, USA) cooled with liquid nitrogen and with the hot jet pulse time set at 250 ms.
Data acquisition was by Agilent – MSD Chem Station ver D.02.00.275 and data elaboration by Hyper Chrom Card ver. 2.4.0 (Thermo Fisher – Rodano, MI, Italy)

GCxGC Operating conditions

Table 1 reports column sets and operative conditions adopted in this study. All columns were from MEGA (Legnano (Milan)-Italy). One micro liter of each sample solution was automatically injected into the GC instrument by an Agilent ALS 7683B under the following conditions: injector: split/splitless in split mode, split ratio: 1/200, injector temperature: 280°C; Carrier gas: Helium.
Temperature programme: from 50°C (1 min) to 280°C (5 min) at 3°C/min. The modulation period was set at 4 s.

Table 1

Acronym	First dimension column (length m x ID mm, ft μm)	Second dimension column (length m x ID mm, ft μm)	p_{in} (KPa)	^1u (cm s^{-1})	^2u (cm s^{-1})	N^1D plates	N^2D plates
Thick OV1701	OV1 - 25x0.25, 0.50	OV1701 - 2.5x0.25, 0.15	132	38.40	124.80	74043	9135
Thick OV17	OV1 - 25x0.25, 0.50	OV17 - 2.5x0.25, 0.15	132	38.40	124.80	74043	9135
Thick OV225	OV1 - 25x0.25, 0.50	OV225 - 2.5x0.25, 0.15	132	38.40	124.80	74043	9135
Thick CW20M	OV1 - 25x0.25, 0.50	CW20M - 2.5x0.25, 0.15	132	38.40	124.80	74043	9135
Thick Cyclodex	OV1 - 25x0.25, 0.50	2,3-DiEthyl-6-TBDMS-β-Cyclodextrin in OV1701 - 2.5x0.25, 0.15	132	38.40	124.80	74043	9135

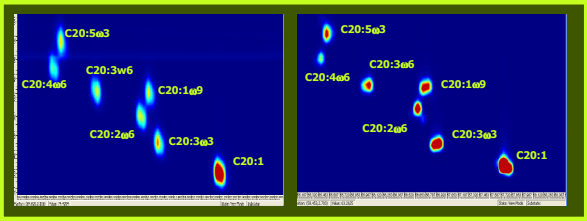

Figure 2: Contour plots of the C:20 FAME cluster (methyl arachidate (C20:0), *cis*-11-eicosenoic acid methyl ester (C20:1w9), *cis*-11,14-eicosadienoic acid methyl ester (C20:2w6), *cis*-8,11,14-eicosatrienoic acid methyl ester (C20:3w6), *cis*-11,14,17-eicosatrienoic acid methyl ester (C20:3w3), methyl arachidonate (C20:4w6), *cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester (C20:5w3)) obtained with different column settings and experimental conditions: 2a- Thick OV1701, 2b- Thick OV225

Results and Discussion

Flow regime optimization

A validated computer programme developed by Beens *et al* [1] to calculate model chromatographic parameters (i.e. 1D and 2D column dimensions and gas flow conditions) was used here to find the best compromise in terms of height equivalent of a theoretical plate (HETP) and column efficiency for both dimensions. The GCxGC column combinations investigated are reported in **Table 1** together with ^{1}D and ^{2}D carrier gas average linear velocities (^1u, ^2u), theoretical number of plates (N) for each dimension (considering a model solute with a capacity factor (k) of 5) based on their measure at vacuum outlet, since a MSD detection was used. A close-to-optimal carrier gas linear velocity was adopted for both dimensions.

[1] J. Beens, H.G. Janssen, M. Adahchour, U.A.Th. Brinkman, J. Chromatogr. A 1086 (2005) 141

Peak Capacity and Separation Measure

Peak Capacity (n) and Separation Measure (S), were adopted to define the metrics of the 2D separation. Peak capacity, n, is an additive quantity based on a constant peak width, and was defined by Giddings as the maximum number of peaks in a selected time interval separated with a given resolution. It was calculated through the following equation:

$$n = \Delta t / w_b \qquad \textbf{Eq1}$$

where Δt is the time interval and w_b is the base peak width that can be assumed to be four times the standard deviation (σ) of the peak.
On the other hand, the separation measure S introduced by Blumberg *et al* [1], is again an additive quantity but it is representative of a separation time interval which is equal to the sum of the separation measures of non-overlapping σ-wide subintervals and, unlike n, S can be used with any shape of chromatographic peaks.

It was calculated using the following equation:

$$S = \Delta t / \sigma_{av} \qquad \textbf{Eq2}$$

where Δt is the arbitrary time interval limited by two peaks a and b, $\Delta t = t_b - t_a$, and σ_{av} is the average σ of a and b, $\sigma_{av} = (\sigma_a + \sigma_b)/2$.
In 2003 Blumberg [2] extended the S concept to a GCxGC separation introducing the S_{GCxGC} that corresponds to the product of the separation measure of each chromatographic dimension. This parameter was here adopted to evaluate the separation power of each GCxGC column combination considering the average **σ** values estimated for a separation of the *n*-alkanes test mixture. The *Experimental* S_{GCxGC} was calculated on C9-C25 separation intervals respectively defined as: $^1\Delta t$ ($^1tC_{25}$ - $^1tC_9$) and $^2\Delta t$ ($^2tC_{25}$ - $^2tC_9$) using the ^{1}D σ_{av} obtained from the analysis of *n*-alkanes with each column combination without modulation and keeping the other chromatographic conditions constant, ^{2}D $\boldsymbol{\sigma}_{av}$ was obtained by analyzing *n*-alkanes with each column combination with a modulation period of 4 s. The component spreading on the chromatographic plane with this homologue series is limited and the $^2\Delta t$ interval is very short influencing the *Experimental* S_{GCxGC} significance. An alternative approach (i.e. *Practical* S_{GCxGC} and n) was therefore adopted extending the ^{2}D time interval to the entire ^{2}D separation time available (obtained by subtracting the ^{2}D dead time, ^{2}D t_M, from the ^{2}D analysis time, 4s) and assuming ^{2}D $\boldsymbol{\sigma}_{av}$ as constant. Experimental S_{GCxGC} and Practical S_{GCxGC} values are reported in **Table 2** together with n measured on the same time intervals and **σ** parameters. S_{GCxGC} values are perfectly comparable with those of "classical" GCxGC column setting (i.e. adopting a narrow bore column in the ^{2}D) and compatible with the suitable separation power for complex samples.

[1] L.M. Blumberg, M.S. Klee, J. Chromatogr. A, 933 (2001) 1
[2] L.M. Blumberg, J. Chromatogr. A, 985 (2003) 29

	Reference Solute n-C9						Reference solute n-C25						Experimental	
Acronym	^{1}D Rt s	^{1}D $pw_{50\%}$	^{1}D σ	^{2}D Rt s	^{2}D $pw_{50\%}$	^{2}D σ	^{1}D Rt s	^{1}D $pw_{50\%}$	^{1}D σ	^{2}D Rt s	^{2}D $pw_{50\%}$	^{2}D σ	S_{GCxGC}	n Peak Capacity
Thick OV1701	386	2.220	0.94	2.00	0.095	0.04	3829	5.100	2.17	3.38	0.095	0.04	75367	4710
Thick OV17	387	2.400	1.02	2.33	0.143	0.06	3829	5.280	2.25	0.43	0.143	0.06	65941	4121
Thick CW20M	380	2.580	1.10	1.38	0.095	0.04	3808	4.920	2.09	2.38	0.143	0.06	42405	2650
Thick OV225	383	2.460	1.05	1.91	0.095	0.04	3823	4.920	2.09	2.24	0.048	0.02	24009	1501
Thick Cyclodex	393	2.520	1.07	3.95	0.238	0.10	3830	5.400	2.30	0.19	0.667	0.28	39803	2492
	Reference Solute n-C9						Reference solute n-C25						Theoretical	
	^{1}D Rt s	^{1}D $pw_{50\%}$	^{1}D σ	^{2}D tm	^{2}D $pw_{50\%}$	^{2}D σ	^{1}D Rt s	^{1}D $pw_{50\%}$	^{1}D σ	^{2}D t_{max}	^{2}D $pw_{50\%}$	^{2}D σ	S_{GCxGC}	n Peak Capacity
Thick OV1701	386	2.220	0.94	0.71	0.095	0.04	3829	5.100	2.17	4.00	0.095	0.04	179549	11222
Thick OV17	387	2.400	1.02	0.71	0.143	0.06	3829	5.280	2.25	4.00	0.143	0.06	113942	7121
Thick CW20M	380	2.580	1.10	0.71	0.095	0.04	3808	4.920	2.09	4.00	0.143	0.06	139511	8719
Thick OV225	383	2.460	1.05	0.71	0.095	0.04	3823	4.920	2.09	4.00	0.048	0.02	236902	14826
Thick Cyclodex	393	2.520	1.07	0.71	0.238	0.10	3830	5.400	2.30	4.00	0.667	0.28	34853	2179

Table 2: ^{1}D retention time (^{1}D tr), ^{1}D peak width at half height (1D pw50%), 1D σ (1D σ), ^{2}D retention time (^{2}D tr), ^{2}D peak width at half height (2D pw50%), 2D σ (^{2}D σ), ^{2}D dead time (^{2}D tm), ^{2}D analysis time ($^2Dt_{max}$), GCxGC Separation Measure (S_{GCxGC}), GCxGC Peak Capacity (n) estimated on the basis of C9 and C25 elution time intervals for each set adopted in this study.

Separation Space Used and Peak Spreading

To investigate the degree of correlation between the two dimensions on the basis of the peak distribution on the chromatographic plane was chosen an approach based on the evaluation of the % of usage of the separation space [1,2] that is a practical measure of the degree of orthogonality, a fundamental aspect for a GCxGC separation. An interesting approach to evaluate the amount of separation space used experimentally was proposed by Ryan et al [1]. This parameter measures the ratio between the area occupied by solute separation and the unused separation space beneath the 2D (i.e. the dead time). **Table 3** reports the amount of separation space used referred to three different model test mixtures: suspected volatile allergens, hydrocarbon and FAME mixtures. Two series of data are reported: the first one, named Separation space used*, includes the wrapped around peaks, i.e. all peaks separated are included, and the lower unusable area of the retention plane is included within the least-retained peak, while the second one excludes them. The net separation space under which data were normalized was referred to 2D column dead time (2D tM) calculated by Poiseuille's law. Experimental data clearly demonstrate that the % of usage of the separation space is maximized even when conventional 1D columns are used in the ^{2}D suggesting that flow regime settings are extremely important and decisive for system orthogonality. A fairy separation of the FAME C_{20} cluster is reported in **Figure 2**.

[1] D. Ryan, P. Morrison, P. Marriott , J. Chromatogr. A 1071 (2005) 47
[2] C. Cordero, P. Rubiolo, B. Sgorbini, M. Galli, C. Bicchi, J. Chromatogr. A 1132 (2006) 26

Table 3: Amount of separation space used and % of usage of the separation space available calculated for the allergens, the Fatty Acids Methyl Esters and hydrocarbons test mixtures.

	1D dimension last eluted(min)	2tM (s)	2D least eluted (s)	2D last eluted (s)	Total retention space usable (s*s)	Retention space used*	Retention space used	% of usage
	ALLERMIX Modulation time 4s Rate 3°/min							
Thick OV1701	50.51	0.71	0.29	3.95	9971	1.11	0.98	98
Thick OV17	50.46	0.71	0.05	3.76	9961	1.13	0.93	93
Thick CW20M	46.80	0.71	0.19	3.81	9238	1.10	0.94	94
Thick OV225	49.86	0.71	0.10	3.95	9842	1.11	0.99	99
Thick Cyclodex	50.44	0.71	0.38	3.86	9957	1.06	0.96	96
	1D elution last eluted(min)	2tM (s)	2D least eluted (s)	2D last eluted (s)	Total retention space usable (s*s)	Retention space used*	Retention space used	% of usage
	HYDROMIX Modulation time 4s Rate 3°/min							
Thick OV1701	27.98	0.71	0.24	3.95	5523	1.13	0.99	99
Thick OV17	27.93	0.71	0.24	3.76	5513	1.07	0.93	93
Thick CW20M	27.83	0.71	0.10	3.95	5494	1.11	0.99	99
Thick OV225	27.83	0.71	0.29	4.00	5494	1.13	1.00	100
Thick Cyclodex	28.26	0.71	0.10	4.00	5579	1.19	1.00	100
	1D elution last eluted(min)	2tM (s)	2D least eluted (s)	2D last eluted (s)	Total retention space usable (s*s)	Retention space used*	Retention space used	% of usage
	FAME MIX Modulation time 4s Rate 3°/min							
Thick OV1701	68.40	0.71	0.52	2.52	13502	0.61	1.55	55
Thick OV17	67.73	0.71	1.10	3.86	13370	0.84	0.96	96
Thick CW20M	67.13	0.71	0.05	3.91	13251	1.17	0.97	97
Thick OV225	67.53	0.71	0.62	2.52	13331	0.58	0.55	55
Thick Cyclodex	67.87	0.71	0.86	2.62	13397	0.53	0.58	58

Conclusions

Experimental data demonstrate that coupling homologous diameter columns, differing in stationary phase and film thickness, each chromatographic dimension can work under a flow regime close to the optimal linear velocity improving both separation power and phase selectivity (confirmed by system orthogonality estimation). In addition, suitable tuning of the elution temperature in combination with a thicker film in the 1D column makes it possible to compensate for the loss of separation efficiency maximizing the peak capacity, to enhance the separation space used and to obtain a suitable number of modulated peaks that ensures a reliable quantitation also for trace analytes. The temperature rate also plays a crucial role in increasing separation efficiency and degree of orthogonality because at lower values it helps to increase the peak spreading in the separation space and enhances the selectivity of the GCxGC system.
Last but not least, homologue diameter column combinations produce a wider 2D peak width improving their compatibility with quadrupole MS detection, when compared to those obtained with a "classical" narrow bore 2D column, and as a consequence its benefits and potential as a GCxGC detector. These topics have already been extensively discussed in previous articles [1,2] and will be the object of a forthcoming publication.

[1] M. Adahchour, M. Brandt, H.U. Baier, R.J.J. Vreuls, A. M. Batenburg, U.A. Th. Brinkman, J. Chromatogr. A 1067 (2005) 245.
[2] C. Cordero, C. Bicchi, D. Joulain, P. Rubiolo, J. Chromatogr. A 1150 (2007) 37

Acknowledgments

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Comprehensive two-dimensional GCxGC with conventional Inner Diameter colums: new column coated with two in series different stationary phases in a single fused silica tubing

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Aims and scope

The optimization of a GCxGC system requires a complex approach since the separation in both dimensions is differently and independently influenced by column dimensions and stationary phases, temperature programming and carrier gas flow rates. In GCxGC, the columns of a set are combined in series and the possibility to apply a close-to-optimal flow regime in both GCxGC dimensions by columns with a conventional ID was recently discussed [1] and the system performance evaluated through conventional chromatographic parameters (i.e. Peak Capacity (*n*) and Separation Measure (*S_1, S_2 and S_{GCxGC}*)) and a parameter specific to GCxGC such as orthogonality. The combination of homologous diameter columns, differing in stationary phase and film thickness, was shown to enable each chromatographic dimension to work with a close to the optimal flow regime resulting in an improved phase selectivity (confirmed by system orthogonality estimation) that partially compensated the loss of efficiency due to wider ^{2}D ID.

A new open tubular capillary column called **DN-UNIQUE™** or **MEGA-2D™** [2] coated in series with different film thickness of two different stationary phases in a single fused silica tubing is here described. This column is here shown to improve GCxGC performance since it avoids the use of unions (press-fits or low dead volume connections) between the first and the second dimension thus eliminating a possible source of leaks and reducing band broadening effects.

The advantages of the single column are here shown through the results of a set of samples including *n*-alkanes (C9-C25), Fatty Acids Methyl Esters (FAME, C4:0-C24:0), hydrocarbons (Boiling Point range 36-254°C), and suspected volatile allergens.

References:
[1] C. Cordero, C. Bicchi, E. Liberto, B. Sgorbini, S. Galli, M. Galli, P. Rubiolo, Proceedings of Dalian International Symposia and Exhibition on Chromatography, June 4-5, 2007, Dalian, China
[2] Patented System by DANI Instruments SpA.

Experimental

Samples

Pure standard samples of **n-alkanes** (from C9 to C25), and **suspected volatile allergens**: limonene, phenylacetaldehyde, linalool, benzyl alcohol, estragole, methyl 2-octynoate, citronellol, geraniol, citral, cinnamic aldehyde, hydroxycitronellal, anisyl alcohol, cinnamic alcohol, eugenol, methyl eugenol, α-isomethylionone, isoeugenol, butylphenyl methylpropional (lilial), coumarine, amyl cinnamic aldehyde, farnesol, amyl cinnamic alcohol, hydroxyisohexyl-3-cyclohexene carboxaldehyde (lyral), hexyl cinnamic aldehyde, benzyl benzoate, benzyl salicylate, benzyl cinnammate, and 1,4-dibromobenzene (ISTD-1), 4,4'-dibromodiphenyl (ISTD-2) were supplied by Sigma-Aldrich (Milan, Italy).
Solvents (cyclohexane, n-hexane, acetone) were all HPLC-grade from Riedel-de Haen (Seelze, Germany). Standard stock solutions and standard working solutions were stored at –18°C.
The **Fatty Acids Methyl Esters mixture** was purchased from Supelco (Milan, Italy) and the hydrocarbons **"Quantitative Reference Standard 512"** mixture (Boiling Point range 36-254°C) was purchased from AC Analytical Controls (Rotterdam, The Netherlands) and consisted of: cyclopentane, n-pentane, cyclohexane, 2,3-dimethylbutane, n-hexane, 1-hexene, methylcyclohexane, 4-methyl-1-hexene, n-heptane, 1,2-dimethylcyclohexane, 2,2,4-trimethylpentane, n-octane, 1,2,4-trimethylcyclohexane, n-nonane, n-decane, n-undecane, n-dodecane, benzene, toluene, trans-decalin, n-tetradecane, ethylbenzene, o-xylene, n-propylbenzene, 1,2,4-trimethylbenzene, 1,2,3-trimethylbenzene, 1,2,4,5-tetramethylbenzene, pentamethylbenzene. Components range from a minimum of 1.1% to a maximum of 5% w/w.
The complex **aroma** sample was supplied by Robertet S.A. Grasse (Cedex) France.

Instrumental set-up

Comprehensive GCxGC/qMS analyses were carried out on a Agilent 6890 GC coupled with a 5975 MS detector (Agilent, Little Falls, DE, USA) operating in E.I. mode at 70 eV. Ion source temperature: 230 °C, Quadrupole temperature 150°C, Transfer line: 280 °C. An automatic tuning was used. Scan range was from 35 m/z to 300 m/z with a scan rate of 10000 amu/s. The system was provided with a two-stage thermal modulator, **Figure 1** (KT 2004 loop modulator from Zoex Corporation, Lincoln, NE, USA) cooled with liquid nitrogen and with the hot jet pulse time set at 250 ms.
Data acquisition was by Agilent – MSD Chem Station ver D.02.00.275 and data elaboration by GC-Image ver 1.8b6s LLC Lincoln (NE) USA.

GCxGC Operating conditions

Table 1 reports column characteristics and operative conditions adopted in this study. All **DN-UNIQUE™** or **MEGA-2D™**. **Figure 2,** columns were from MEGA (Legnano (Milan)-Italy).
One micro liter of each sample solution was automatically injected into the GC instrument by an Agilent ALS 7683B under the following conditions: injector: split/splitless in split mode, split ratio: 1/200, injector temperature: 280°C; Carrier gas: Helium.
Temperature programme: from 50°C (1 min) to 280°C (5 min) at 3°C/min. The modulation period was set at 4 s.

Acronym	First dimension column (length m x ID mm, ft µm)	Modulation loop dimensions (length m x ID mm, ft µm)	Connections	Second dimension column (length m x ID mm, ft µm)
Thick OV1701	OV1 - 20x0.25, 0.50	deactivated 1.0x0.25, 0.15	press-fits	OV1701 - 2.5x0.25, 0.15
Thick OV17	OV1 - 20x0.25, 0.50	deactivated 1.0x0.25, 0.15	press-fits	OV17 - 2.5x0.25, 0.15
MEGA-2D™ OV1_OV1701	OV1 - 20x0.25, 0.50	deactivated 1.0x0.25, 0.15	none	OV1701 - 2.5x0.25, 0.15
MEGA-2D™ OV1_OV17	OV1 - 20x0.25, 0.50	deactivated 1.0x0.25, 0.15	none	OV17 - 2.5x0.25, 0.15

Table 1

Acronym	p_{in} (KPa)	^1u (cm s^{-1})	^1t_R (s)	^2u (cm s^{-1})	^2t_R (s)	N^1D plates	N^2D plates
Thick OV1701	180	45.85	50.93	151.85	0.71	79826	14683
Thick OV17	180	45.85	50.93	151.85	0.71	79826	14683
MEGA-2D™ OV1_OV1701	180	45.85	50.93	151.85	0.71	79826	14683
MEGA-2D™ OV1_OV17	180	45.85	50.93	151.85	0.71	79826	14683

Figure 1

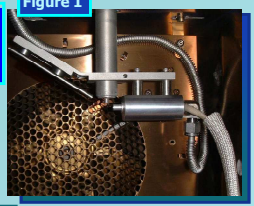
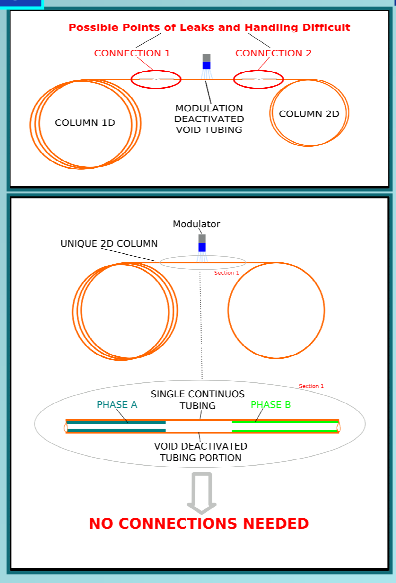


Figure 2



Results and Discussion

Peak Capacity and Separation Measure

Peak Capacity (*n*) and Separation Measure (*S*), were adopted to evaluate the separation power of the DN-UNIQUE™ or MEGA-2D™ using the average σ values obtained with the separation of the *n*-alkanes test mixture.

Peak capacity (*n*), was defined by Giddings as the maximum number of peaks in a selected time interval separated with a given resolution. It was calculated through the following equation:

$$n = \Delta t / w_b \qquad \text{Eq1}$$

where Δt is the time interval and w_b is the base peak width that can be assumed to be four times the standard deviation (σ) of the peak.
The separation measure *S* introduced by Blumberg *et al* [1], is again an additive quantity but it is representative of a separation time interval which is equal to the sum of the separation measures of non-overlapping σ-wide subintervals that can be used with any shape of chromatographic peaks. It was calculated using the following equation:

$$S = \Delta t / \sigma_{av} \qquad \text{Eq2}$$

where Δt is the arbitrary time interval limited by two peaks a and b, $\Delta t = t_b - t_a$, and σ_{av} is the average σ of a and b, $\sigma_{av} = (\sigma_a + \sigma_b)/2$.
In 2003 Blumberg [2] extended the *S* concept to a GCxGC separation introducing the S_{GCxGC} that corresponds to the product of the separation measure of each chromatographic dimension.

The ***Experimental* S_{GCxGC}** was calculated on C9-C25 separation intervals respectively defined as: ^1Dt ($^1tC_{25}$ - $^1tC_9$) and ^2Dt ($^2tC_{25}$ - $^2tC_9$) using the ^{1}D σ_{av} was obtained from the analysis of *n*-alkanes with each column without modulation and keeping the other chromatographic conditions constant, ^{2}D $\boldsymbol{\sigma}_{av}$ was measured by analyzing *n*-alkanes with each column combination with a modulation period of 4 s.
Table 2 reports *n* values and *Experimental* S_{GCxGC} measured on the same time intervals and **σ** parameters.
S_{GCxGC} values are perfectly comparable to those of "classical" GCxGC column setting (i.e. with a narrow bore column in the ^{2}D) and compatible with a separation power suitable for complex samples.

[1] L.M. Blumberg, M.S. Klee, J. Chromatogr. A, 933 (2001) 1
[2] L.M. Blumberg, J. Chromatogr. A, 985 (2003) 29

Table 2: ^{1}D absolute retention time (^1t_R), ^{1}D σ (^{1}D σ), ^{2}D absolute retention time (^2t_R), ^{2}D σ (^{1}D σ), ^{2}D hold-up time (^2t_M), ^{2}D analysis time ($^2t_{max}$), ^{1}D Separation Measure (S_1), ^{2}D Separation Measure (S_2), GCxGC Separation Measure (S_{GCxGC}), GCxGC Peak Capacity (*n*) estimated on the basis of C_9 and C_{25} elution time intervals for each set.

	Reference solute *n*-C_9				Reference solute *n*-C_{25}				Practical			
	^1t_R (s)	^1D σ (s)	^2t_M (s)	^2D σ (s)	^1t_R (s)	^1D σ (s)	$^2t_{MAX}$ (s)	^2D σ (s)	S_1	S_2	S_{GCxGC}	*n*
Thick OV1701	398	0.94	0.59	0.04	3580	2.17	4.00	0.06	2043	84	171992	10749
Thick OV17	394	1.02	0.59	0.06	3598	2.25	4.00	0.06	1961	56	109956	6872
MEGA-2D™ OV1_OV1701	380	0.91	0.59	0.04	3579	2.09	4.00	0.03	2130	93	197086	12318
MEGA-2D™ OV1_OV17	383	1.00	0.59	0.04	3574	2.09	4.00	0.06	1363	68	139379	8711

Separation Space Used and Peak Spreading

The % of usage of the separation space [1,2] was used to investigate the degree of correlation between the two dimensions on the basis of the peak distribution on the chromatographic plane. This parameter was proposed by Ryan et al [1] and is a practical measure of the degree of orthogonality, a fundamental aspect for a GCxGC separation. It measures the ratio between the area occupied by solute separation and the unused separation space beneath the ^{2}D dead time. **Table 3** reports the amount of separation space used referred to three different model test mixtures: suspected volatile allergens, hydrocarbon and FAME mixtures. Two series of data are reported: the first one, called **Separation space used***, includes the wrapped around peaks, i.e. all separated peaks are included, and the lower unused area of the retention plane is included within the least-retained peak. The second one excludes them. The net separation space through which data were normalized, was referred to ^{2}D column dead time (^{2}D t_M) calculated by Poiseuille's law. **Experimental data clearly show that the % of usage of the separation space is maximized even if conventional i.d. columns are used. A fairy separation of the suspected allergens standard mixture is reported in Figure 3.**

[1] D. Ryan, P. Morrison, P. Marriott , J. Chromatogr. A 1071 (2005) 47
[2] C. Cordero, P. Rubiolo, B. Sgorbini, M. Galli, C. Bicchi, J. Chromatogr. A 1132 (2006) 26

	^1D last eluted (min)	^2t_M (s)	^2D least eluted (s)	^2D last eluted (s)	Total available separation space (s^2)	Separation space used*	Separation space used	% of usage
FAME test mixture								
Thick OV1701	44.67	0.59	0.29	3.95	9139	1.07	0.99	99
Thick OV17	46.78	0.59	0.05	3.76	9571	1.09	0.93	93
MEGA-2D™ OV1_OV1701	43.47	0.59	0.56	3.96	8893	1.00	0.99	99
MEGA-2D™ OV1_OV17	45.00	0.59	0.48	3.88	9207	1.00	0.96	96
Hydrocarbons test mixture								
Thick OV1701	22.45	0.59	0.24	3.95	4593	1.09	0.99	99
Thick OV17	22.67	0.59	0.24	3.76	4638	1.03	0.93	93
MEGA-2D™ OV1_OV1701	22.33	0.59	0.21	3.88	4569	1.08	0.96	96
MEGA-2D™ OV1_OV17	22.93	0.59	0.08	3.96	4692	1.14	0.99	99
Suspected allergens test mixture								
Thick OV1701	44.67	0.59	0.29	3.95	9139	1.07	0.99	99
Thick OV17	46.78	0.59	0.05	3.76	9571	1.09	0.93	93
MEGA-2D™ OV1_OV1701	43.47	0.59	0.56	3.96	8893	1.00	0.99	99
MEGA-2D™ OV1_OV17	45.00	0.59	0.48	3.88	9207	1.00	0.96	96

* included peaks beneath the ^{2}D hold-up time

Table 3: Amount of separation space used and % of usage of the separation space available calculated for the allergens, the Fatty Acids Methyl Esters and hydrocarbons test mixtures.

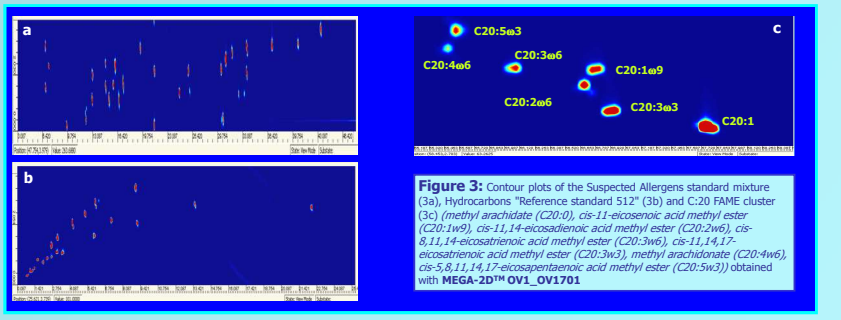


Figure 3: Contour plots of the Suspected Allergens standard mixture (3a), Hydrocarbons "Reference standard 512" (3b) and C:20 FAME cluster (3c) *(methyl arachidate (C20:0), cis-11-eicosenoic acid methyl ester (C20:1w9), cis-11,14-eicosadienoic acid methyl ester (C20:2w6), cis-8,11,14-eicosatrienoic acid methyl ester (C20:3w6), cis-11,14,17-eicosatrienoic acid methyl ester (C20:3w3), methyl arachidonate (C20:4w6), cis-5,8,11,14,17-eicosapentaenoic acid methyl ester (C20:5w3))* obtained with **MEGA-2D™ OV1_OV1701**

Conclusions

Experimental data demonstrate that the two **MEGA-2D™ or DN-UNIQUE™** columns tested, with 0.25 mm homologous diameter coated with different film thickness of two stationary phases in series corresponding to the two chromatographic dimensions , operating with a flow regime close to the optimal linear velocity, **gives high separation power and phase selectivity (confirmed by system orthogonality estimation).** Moreover, a suitable tuning of the elution temperature in combination with a the choice of a suitable thicker film in the ^{1}D column makes it possible:
a) to compensate the loss of separation efficiency, even because the increase in elution temperature results in a narrower 2D peak width and a higher peak capacity,
b) to enhance the separation space used and
c) to obtain a suitable number of modulated peaks enabling a reliable quantitation also for trace analytes. Last but not least, homologue diameter column combinations improve peak compatibility with quadrupole MS detection giving a wider ^{2}D peak width, when compared to those obtained with a "conventional" narrow bore ^{2}D column.

Acknowledgments

This research was carried out within the project entitled: "Sviluppo di metodologie innovative per l'analisi di prodotti agroalimentari" (FIRB Cod.: RBIP06SXMR_002) of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) (Italy).

A new column coated with two in series different stationary phases in a single fused silica tubing of conventional inner diameter for Comprehensive Two-Dimensional GCxGC

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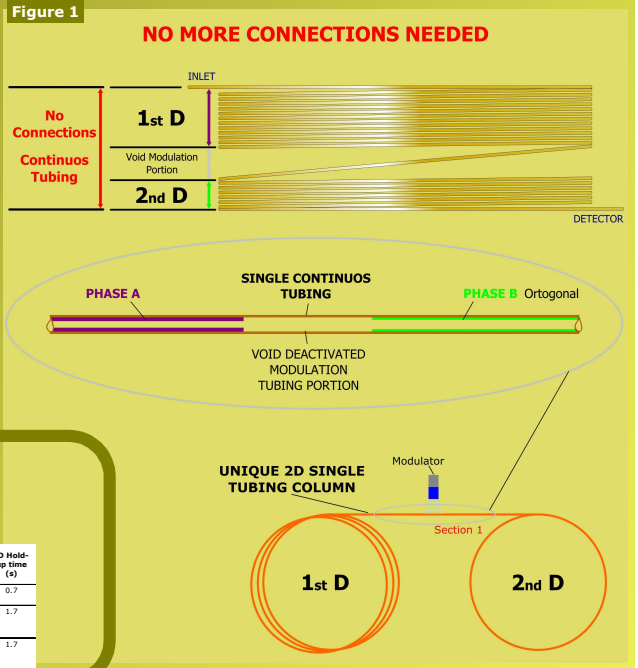
Aims and scope

Several parameters influence the optimization of a GCxGC system including column dimensions and stationary phases, temperature programming and carrier gas flow rates. The use of two conventional ID columns in both GCxGC dimensions affording a close-to-optimal flow regime was recently discussed [1] by evaluating Peak Capacity (*n*), Separation Measure (*S1, S2* and S_{GCxGC}) and orthogonality. This combination provided an improved phase selectivity compensating the loss of efficiency due to wider ^{2}D ID.

A new open tubular capillary column [2], called **DN-UNIQUE™** or **MEGA-2D™** coated with two different stationary phases (differing in composition and film thickness) in a single fused silica tubing is here presented.
This column is an effective GCxGC improvement since it avoids unions between the two dimensions thus eliminating possible leaks and reducing band broadening effects.
The performance of this column is here compared to that of a conventional (^{1}D 0.25 mm ID and ^{2}D 0.10 mm ID) column set up. **DN-UNIQUE™** or **MEGA-2D™** peak capacity, orthogonality, peak area reproducibility and linearity have been here evaluated through the analysis of two test mixtures (Fatty Acids Methyl Esters and volatile suspected allergens) and through a target analysis approach aimed at quantifying volatile suspected allergens in medium-complexity fragrances.

[1] C. Cordero, C. Bicchi, P. Rubiolo, M. Galli, S. Galli. 59th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1-7, 2008 New Orleans, Luisiana, USA
[2] Patent pending by DANI Instruments SpA and MEGA snc

Figure 1



Experimental

Samples
Pure standard samples of **volatile suspected allergens** were supplied by Sigma-Aldrich (Milan, Italy).
Solvents (cyclohexane, n-hexane, acetone) were all HPLC-grade from Riedel-de Haen (Seelze, Germany).
The **Fatty Acids Methyl Esters mixture** was purchased from Supelco (Milan, Italy).
The **fragrance** sample was supplied by Robertet S.A. Grasse (Cedex) France.

Instrumental Set-up
Comprehensive GCxGC/MS analyses were carried out on a Agilent 6890 GC coupled with a 5975 MS detector (Agilent, Little Falls, DE, USA) operating in E.I. mode at 70 eV. Ion source temperature: 230 °C, Quadrupole temperature 150°C, Transfer line: 280 °C. An automatic tuning was used. Scan range was from 35 m/z to 300 m/z with a scan rate of 10000 amu/s. The system was provided with a two-stage thermal modulator (KT 2004 loop modulator from Zoex Corporation, Lincoln, NE, USA) cooled with liquid nitrogen and with the hot jet pulse time set at 250 ms. Data acquisition was by Agilent – MSD Chem Station ver D.02.00.275 and data elaboration by GC-Image ver 1.8b6s LLC Lincoln (NE) USA.

GCxGC Operating conditions
Table 1 reports column characteristics and operative conditions adopted in this study. All **DN-UNIQUE™** or **MEGA-2D™**, **Figure 1**, columns were from MEGA (Legnano (Milan)-Italy).
One micro liter of each sample solution was automatically injected into the GC instrument by an Agilent ALS 7683B under the following conditions: injector: split/splitless in split mode, split ratio: 1/200 or 1/20, injector temperature: 280°C; Carrier gas: Helium.
Temperature programme: from 50°C (1 min) to 280°C (5 min) at 3°C/min. The modulation period was set at 4 s.

Table 1

Column Set N°	First dimension column (length m x ID mm, df µm)	Second dimension column (length m x ID mm, df µm)	P_{in} (KPa)	^1u (cm s^{-1})	^2u (cm s^{-1})	^{2}D Hold-up time (s)
1	OV1 - 25x0.25, 0.15	OV1701 - 1.0x0.10, 0.10 (loop 1.0x0.10)	298	15	147	0.7
2	MEGA-2D™ OV1_OV1701 OV1 - 25x0.25, 0.50 OV1701 - 2.5x0.25, 0.15 (loop 1.0x0.25)		175	48	149	1.7
3	MEGA-2D™ OV1_OV17 OV1 - 25x0.25, 0.50 OV17 - 2.5x0.25, 0.15 (loop 1.0x0.25)		175	48	149	1.7

Results

Basic Performance: *Peak Capacity*

GCxGC net Peak Capacity ($n_{c\ GCxGC}$) was adopted to evaluate the separation power of the DN-UNIQUE™ or MEGA-2D™.

Peak capacity (*n*), defined by Giddings as the maximum number of peaks in a selected time interval separated with a given resolution, was calculated for each chromatographic dimension through the equation:

$$n = \Delta t / w_b$$

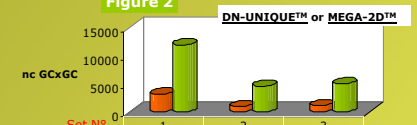
where Δt is the time interval, w_b is the base peak width assumed to be four times the standard deviation (σ) of the peak.
Net peak GCxGC capacity ($n_{c\ GCxGC}$) is the product of *n* calculated for each chromatographic dimension. It was referred to the separation intervals between the first and the last eluted compound of the two test mixtures in the ^{1}D, i.e. $^1D = (^1t_{C_{last\ eluted}} - {}^1t_{C_{first\ eluted}})$, and for the ^{2}D it was comprised between the hold-up and the total analysis time, i.e. $^2D = (^2t_{analysis\ time} - {}^2t_{hold\text{-}up\ time})$. ^{1}D σ_w was estimated by analyzing test mixtures without modulation and keeping the other chromatographic conditions constant while ^{2}D $\boldsymbol{\sigma_t}$ with a modulation period of 4 s.

Table 2 reports ^{1}D σ and ^{2}D σ calculated for the first and the last eluted components of the two test mixtures while **Figure 2** reports $n_{c\ GCxGC}$ values calculated for the column set under study.

Table 2

	Benzyl alcohol		*Benzyl Cinnammate*		
Set N°	$^1D\sigma$ (s)	$^2D\sigma$ (s)	$^1D\sigma$ (s)	$^2D\sigma$ (s)	$n_{c\ GCxGC}$
1	1.81	0.08	2.29	0.08	3214
2	1.53	0.20	2.32	0.18	952
3	1.53	0.16	2.32	0.17	1112
	Methyl hexanoate		*Methyl tetracosanoate*		
Set N°	$^1D\sigma$ (s)	$^2D\sigma$ (s)	$^1D\sigma$ (s)	$^2D\sigma$ (s)	$n_{c\ GCxGC}$
1	2.04	0.03	2.30	0.03	11961
2	1.66	0.09	1.79	0.05	4581
3	1.66	0.08	1.79	0.04	5056

Figure 2

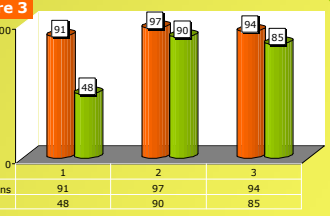


Set N°	1	2	3
Volatile Suspected allergens	3214	952	1112
FAME	11961	4581	5056

Basic Performance: *Separation Space Used*

The **% of usage of the separation space** [1,2] was used to investigate the degree of correlation between the two dimensions on the basis of the peak distribution on the chromatographic plane. This parameter is a practical measure of the degree of orthogonality and indicates the ratio between the area occupied by solute separation and the unused separation space beneath the ^{2}D dead time. **Figure 3** reports the amount of separation space used referred to volatile suspected allergens and FAME test mixtures.
The net separation space through which data were normalized, was referred to ^{2}D column hold-up time (^{2}D t_M).

Figure 3

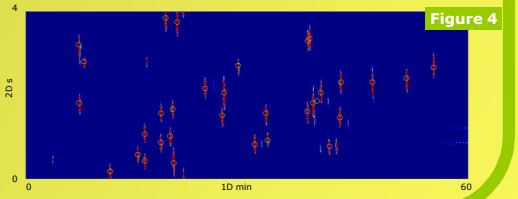


	1	2	3
Volatile Suspected allergens	91	97	94
FAME	48	90	85

Experimental data show that the % of usage of the separation space is maximized with conventional ID columns, DN-UNIQUE™ or MEGA-2D™, because of the improved exploitation of the ^{2}D stationary phase. A fairy separation of the suspected allergens standard mixture is reported in Figure 4.

[1] D. Ryan, P. Morrison, P. Marriott , J. Chromatogr. A 1071 (2005) 47
[2] C. Cordero, P. Rubiolo, B. Sgorbini, M. Galli, C. Bicchi, J. Chromatogr. A 1132 (2006) 26

Figure 4



Target Analysis: *Precision and Linearity*

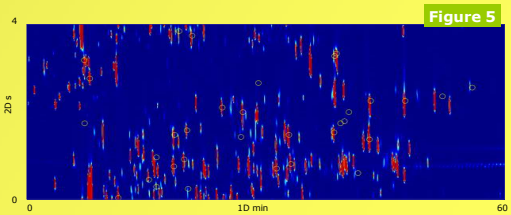
Volatile suspected allergen quantitative determination is part of the fragrance **quality control assessment** and should take into account the highly variable distribution of fragrance components and adopt experimental conditions to compensate peak distortions due to both column overloading and strong retention effects. Widely used orthogonal stationary phase combinations, such as ^{1}D OV1/^{2}D CW20M, were not effective enough in particular with the polar analytes affected by a strong ^{2}D retention due to the inappropriate elution temperature of the ^{2}D column. These effects are well overcome by the adoption of OV1701 or OV17 but the higher correlation (low orthogonality) between the two dimensions for the 0.25/0.10 mm ID column settings need for slower temperature rates, and consequently, higher analysis times.
The exploitation of ^{2}D stationary phase selectivity showed by 0.25 mm homologous ID column combination, resulting in significant peak spreading over the chromatographic plane, and the higher ^{2}D column loadability suggested to test **DN-UNIQUE™ or MEGA-2D™** for an effective separation of target allergens and to evaluate if their peak capacity was high enough to separate target analytes in a medium complexity fragrance.
Correlation coefficients (R^2) estimated by regression analyses over a 50-2 mg/L range and **precision** results (expressed as RSD%) referred to the 2D area of each analyte measured over six replicates, are reported in **Table 3**.

Table 3

		Regression analyses R2		2D Area Precision RSD%	
Component Name	SIM m/z ions	Set N° 1	MEGA-2D	Set N° 1	MEGA-2D
amylcinnamic aldehyde	**202**, 201, 129	0.992	0.996	2.05	2.03
anisyl alcohol	**138**, 137, 109	0.973	0.994	2.15	1.93
benzyl alcohol	**108**, 79, 107	1.000	0.997	2.61	0.52
benzyl benzoate	**105**, 212, 194	0.997	0.990	2.05	1.62
benzyl salicylate	**91**, 228, 65	0.999	0.994	1.64	0.93
cinnamic alcohol	**92**, 134, 115	0.980	0.999	1.37	2.60
cinnamic aldehyde	**131**, 132, 103	0.991	0.998	5.54	4.59
coumarine	**146**, 118, 89	0.996	0.998	2.05	3.21
farnesol isomer I	**69**, 93, 81	0.986	0.999	2.15	2.15
farnesol isomer II	**69**, 93, 81	0.988	0.999	2.61	2.06
geraniol	**69**, 123, 93	0.991	0.998	2.05	2.05
hexylcinnamic aldehyde	**216**, 215, 129	0.980	0.999	1.64	1.62
limonene	**164**, 149, 131	0.999	0.998	1.37	1.36
linalool	**68**, 93, 67	0.999	0.999	5.54	1.98
phenylacetaldehyde	**91**, 120, 92	0.995	0.997	1.77	3.52

Results show that MEGA-2D™ OV1_OV1701 operating at controlled flow, temperature and modulation period conditions, can successfully be used for the target analysis of volatile suspected allergens in medium complexity fragrance (see Figure 5).
MEGA-2D™ is characterized by an improved exploitation of stationary phase selectivity and an increased 2D column loadability; its limited net peak capacity, if compared with an equivalent conventional column setting limits its use to samples with medium complexity.

Figure 5



Conclusions

Experimental data demonstrate that the two **MEGA-2D™ or DN-UNIQUE™** columns tested, with 0.25 mm homologous diameter coated with different film thickness of two stationary phases in series operating with a flow regime close to the optimal linear velocity, could successfully be used for specific applications. This unique columns avoids unions between the two dimensions and, as a consequence, possible leaks and band broadening effects.
Their performances were verified through the definition of several method performance parameters in a target analysis approach for suspected allergens quantification in medium complexity fragrances. Linearity over the working range and precision were, above all, issues of interest even if compared with GCxGC separations performed with conventional column combinations. Despite their reduced peak capacity the proper exploitation of 2D stationary phase can compensate this loss giving reliable results in shorter analysis times.

Acknowledgments

This research was carried out within the project entitled: "Sviluppo di metodologie innovative per l'analisi di prodotti agroalimentari" (FIRB Cod.: RBIP06SXMR_002) of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) (Italy).