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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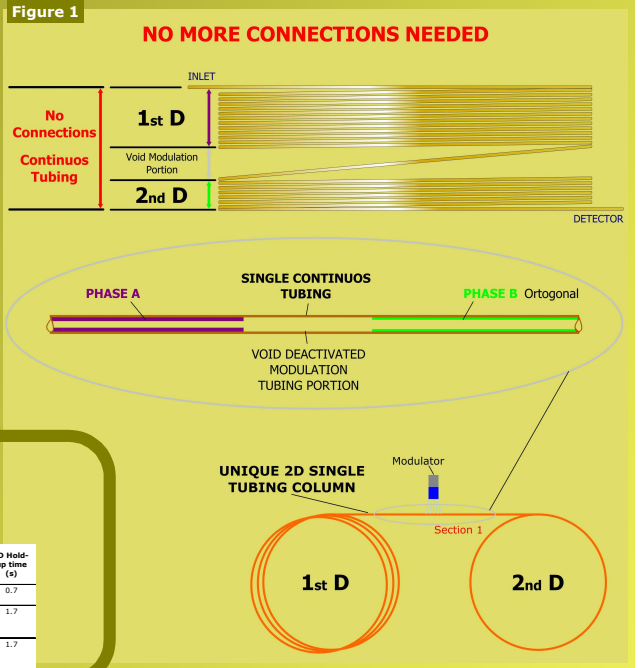
Aims and scope

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:

$$n = \Delta t / w_b$$

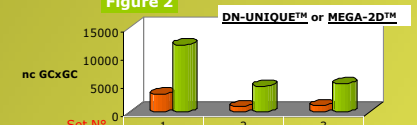
where Δt is the time interval, w_b is the base peak width assumed to be four times the standard deviation (σ) of the peak.
Net peak GCxGC capacity ($n_{c\ GCxGC}$) is the product of *n* calculated for each chromatographic dimension. It was referred to the separation intervals between the first and the 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

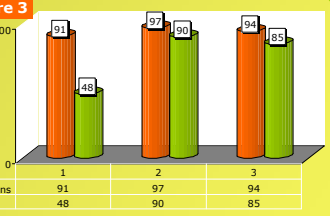


Set N°	1	2	3
Volatile Suspected allergens	3214	952	1112
FAME	11961	4581	5056

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

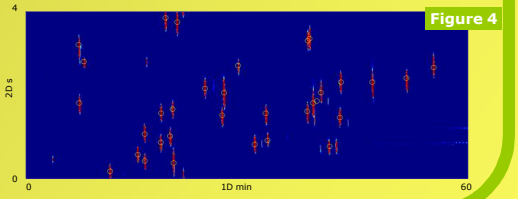


	1	2	3
Volatile Suspected allergens	91	97	94
FAME	48	90	85

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*

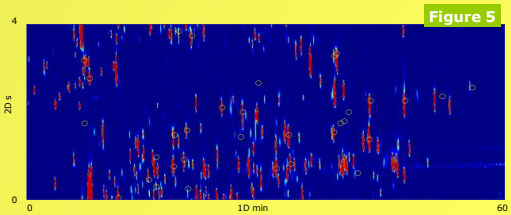
Volatile suspected allergen quantitative determination is part of the fragrance **quality control assessment** and should take into account the highly variable distribution of fragrance components and adopt experimental conditions to compensate peak distortions due to both column overloading and strong 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

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