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

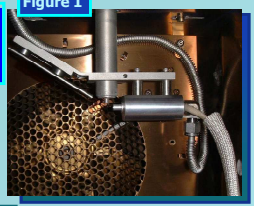
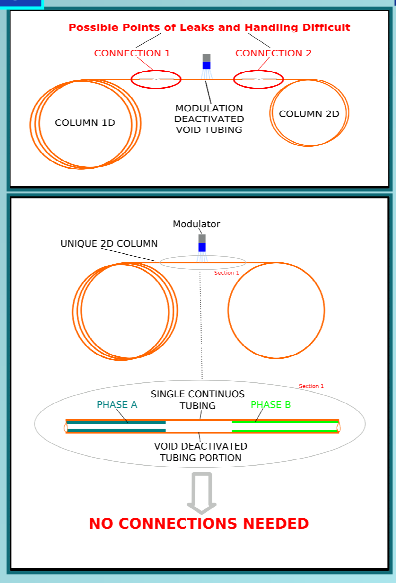


Figure 2



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.

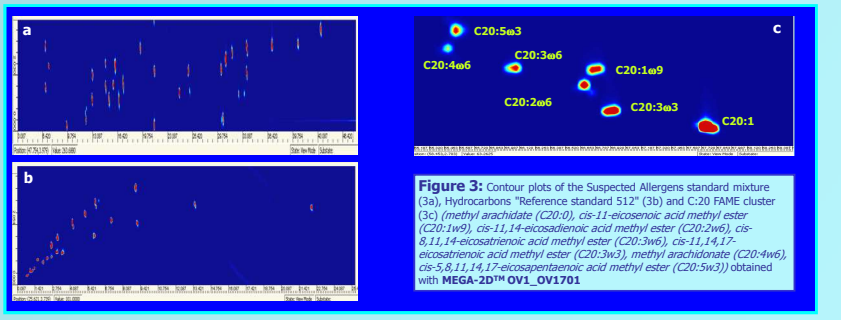


Figure 3: Contour plots of the Suspected Allergens standard mixture (3a), Hydrocarbons "Reference standard 512" (3b) and C:20 FAME cluster (3c) *(methyl arachidate (C20:0), cis-11-eicosenoic acid methyl ester (C20:1w9), cis-11,14-eicosadienoic acid methyl ester (C20:2w6), cis-8,11,14-eicosatrienoic acid 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

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