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.

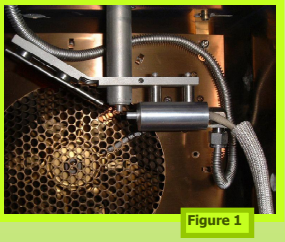

Figure 1

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

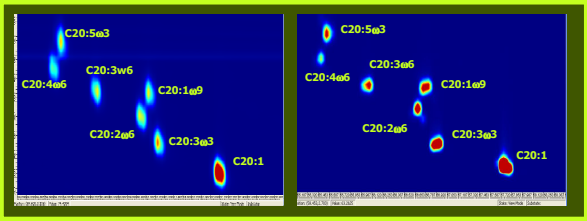

Figure 2: Contour plots of the C:20 FAME cluster (methyl arachidate (C20:0), *cis*-11-eicosenoic acid methyl ester (C20:1w9), *cis*-11,14-eicosadienoic acid methyl ester (C20:2w6), *cis*-8,11,14-eicosatrienoic acid methyl ester (C20:3w6), *cis*-11,14,17-eicosatrienoic acid methyl ester (C20:3w3), methyl 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

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