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High-speed gas chromatography with direct resistively-heated column (ultra fast module-GC)-separation measure (*S*) and other chromatographic parameters under different analysis conditions for samples of different complexities and volatilities

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Abstract

The influence of GC speed on the separation capability of a chromatographic system is reported measuring a series of parameters including separation measure (S), peak capacity (n), peak width (w), analysis time, t_b (determined on the last eluting compound) and separation measure/analysis time ratio (S/t_b) determined by analyzing a bergamot essential oil sample and a standard mixture of pesticides. Conventional GC, fast GC (with 10 m (FGC10) and 5 m (FGC5) narrow-bore columns), and direct resistively-heated ultra fast module-GC (UFM-GC) were the GC speed approaches used. The influence of different heating rates with a constant flow for FGC5, FGC10, and UFM-GC and with variable flows for UFM-GC on S, n, w, S/t_b, and t_b was also studied.

The results of this study show that:

(a) separation capability of the chromatographic system (i.e. S and n) and analysis time depend on the GC approaches. Within each GC approach, S and n and analysis time depend on the heating rates, although to a different extent, and S and n decrease much less than the gain in analysis time, in particular when fast heating rates are applied;

(b) in UFM-GC, the loss of separation capability with heating rate can also be partially compensated by the choice of an appropriate flow rate that, within each heating rate, may contribute to increase S while reducing t_b;

(c) within a specific GC approach, the chromatographic system (column and stationary phase) and conditions (heating and flow rates) must be such to achieve a suitable S-value when two analytes must be separated with a given resolution in a minimum analysis time.

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1. Introduction

The technological innovations in GC instrumentation (electronic pressure control of the mobile phase, high frequency FID detectors, dedicated software) of the last decade have greatly contributed to making high-speed capillary GC popular even in routine analysis. Several authors critically reviewed different topics concerning high-speed GC as such or in combination with MS including theory and speed of GC analyses [1–5], and practical approaches to high-speed GC and GC–MS [6–9].

The speed of a GC analysis mainly depends on column inner diameter (i.d.) and length and analysis conditions (heating and flow rates). Mc Nair and Reed [10] and Blumberg and Klee [11] investigated and discussed in depth the influence of heating rate on the speed of a GC analysis with columns with different i.d. and length. On the basis of the classification proposed by Blumberg and Klee [2] by peak width, and later integrated by Magni et al. [5] with column heating rate, GC nowadays offers a number of approaches as regards the speed of analysis: conventional, fast, super-fast, and ultra

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fast-GC. Such a number of GC approaches, however, makes it necessary to adopt a metric of the GC separation in order to evaluate how the speed may influence the separation capability of a chromatographic system for a given analysis. The parameters currently used to define the metrics of a GC separation are resolution, R (separation of two peaks), separation number, SN (the number of well-separated peaks within any homologue pair), peak capacity, n (the maximum number of peaks that can be separated by a chromatographic system with a given resolution), and the separation measure, S, a universal parameter recently introduced by Blumberg and Klee and defined as the number of consecutive non-overlapping σ-intervals within an arbitrary time interval $t_a - t_b$ [12].

These parameters can be calculated through the classic equations:

$$R = \frac{t_{R2} - t_{R1}}{w_b} \quad (1)$$

$$SN = \frac{t_{R2} - t_{R1}}{2w_h + 1} \quad (2)$$

$$n = \frac{\Delta t}{w_b} \quad (3)$$

$$S = \frac{\Delta t}{\sigma_{av}} \quad (4)$$

where in Eq. (1) t_{R2} and t_{R1} are the retention times of the two components considered and w_b is the average peak width at base line, $w_b = (w_{b2} + w_{b1})/2$, in Eq. (2) w_h is the average half-height peak width, in Eq. (3) Δt is the time interval and w_b is the average base peak width, and in Eq. (4) 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 the two peaks a and b, $\sigma_{av} = (\sigma_a + \sigma_b)/2$. Eqs. (3) and (4) are approximated for programmed temperature analysis where peak width is almost constant over the whole analysis (Eq. (3)) and σ variation (Eq. (4)) is in the limits indicated by Blumberg and Klee [12].

When introducing the separation measure (S), Blumberg and Klee discussed in depth the limits of R, SN and n, in particular emphasizing their incompatibility and lack of additivity [12]. These limits were mainly due to the fact that: (1) R measures the separation of two neighboring peaks, and as a consequence, can only be considered as a local metric of separation; (2) SN, gives the number of well-separated peaks within any homologue pairs, and can be viewed as a regional metric of separation; and (3) n gives the approximate maximum number of peaks separable with a given resolution on a given column and is a global metric of separation. As mentioned above, R, SN and n are based on different peak width metrics: w_b for R, $2w_h$ for SN, and $w_b = 4\sigma$ for n, making their comparison and/or correlation difficult. Moreover, R and SN are not additive, thus preventing the estimation of the actual number of peaks potentially separable by a column in a predetermined separation region. 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; several different and not always compatible equations have been advanced for its calculation, and the Lan and Jorgenson equation has been chosen here, because it overcomes some of the limits of the others [13–15]. On the other hand, the separation measure S is an additive quantity that, by definition, includes all the previous parameters because it is representative of a separation time interval which is equal to the sum of the separation measures of all of its non-overlapping σ-wide subintervals. Last but by no means least, unlike the other parameters, S can be used with any shape of chromatographic peaks.

In a recent article direct resistively-heated column-gas chromatography (Ultra fast module-GC, UFM-GC) was applied to high-speed GC analysis of essential oils of differing complexities and the results compared to those obtained by GC with conventional inner diameter (i.d.) columns (0.25 mm) of different lengths (5 and 25 m long) and by fast GC (FGC) with narrow-bore columns (0.1 mm i.d., 5 m long) [16]. UFM-GC was carried out through direct resistive-heating of the capillary columns in order to achieve reliable temperature programming rates of 1–20 °C/s. The ultra fast module adopted here was that described by Magni et al. [5] and derived from Overton's system [17] for heating very short narrow-bore columns (1–2 m) for portable μFast GCs [18], and modified in agreement with Mustacich's patents [19–22] to extend its use to capillary columns with a broad range of lengths and diameters and enabling it to be assembled inside a conventional GC oven. The system adopted incorporates heating and temperature-sensing elements distributed along the column.

This study aimed to evaluate how GC approaches (conventional GC, fast GC with 10 m (FGC10) and 5 m (FGC5) narrow-bore columns, and direct resistively-heated ultra fast module-GC) and analysis conditions (heating and flow rates) influence the separation capability of a chromatographic system. The Blumberg and Klee's separation measure S was adopted as a descriptor of the metric of separation of the analysis of a bergamot essential oil sample and a standard mixture of pesticides. S was also used to compare the separation capability of GC systems producing different speed of analysis and to evaluate UFM-GC performance through a model of metric of separation. In addition to S, analysis time, t_b (determined on the last eluting compound), separation measure/analysis time ratio (S/t_b), peak capacity (n) and peak width (w) were also determined. To the best of the authors knowledge, this is one of the first times in which S is used to investigate these topics and applied to the analysis of real world samples.

2. Experimental

2.1. Samples

A solution of bergamot essential oil obtained by diluting 5 mg in 1 mL of cyclohexane (1:200) and a standard solution

Table 1
Characteristics of the columns used in the present study

Stationary phase	OV-1701			
GC approach	Conventional GC	FGC10	FGC5	UFM-GC
Length (m)	25	10	5	5
Internal diameter (mm)	0.25	0.1	0.1	0.1
Film thickness (μm)	0.3	0.3	0.1	0.1

of pesticides containing 0.1 mg/mL of α-HCH, γ-HCH, heptachlor, chlortalonil, parathion–methyl, malathion, fenitrothion, α-endosulfan, chlordane *trans*, chlordane *cis*, dieldrin, *o*,*p*′-DDT, β-endosulfan, *p*,*p*′-DDT were automatically injected into the GC instruments. Injected volume: 1 μL.

2.2. GC analysis

GC analyses were carried out on a Thermo Electron Trace GC unit (Rodano, Italy) and a Thermo Electron Trace 2000 unit provided with the ultra fast GC option including the UFM-GC column module incorporating a directly resistively-heated capillary column providing temperature programming rates up to 20 °C/s. Both systems were fitted with AI 3000 automatic injector (Thermo Finnigan, Rodano, Italy) and high frequency fast FID detector (300 Hz, time constant: 6 ms). Data processing was by Chrom-card software (version 2.01-32 bit) (Thermo Electron, Rodano, Italy).

2.3. Columns

All analyses were carried out using OV-1701 as stationary phase. A series of FSOT-high temperature silylated columns of different length and i.d. were used; Table 1 reports the characteristics of the columns. All columns were from MEGA (Legnano, Italy). UFM-GC column modules with direct resistive-heating were from Thermo Electron, Rodano, Italy.

2.4. GC conditions

Bergamot essential oil and pesticide standard solution were analyzed by applying the GC speed approaches listed above (conventional GC, FGC10, FGC5, UFM-GC) under different temperature rates and flow conditions. Table 2 reports heating and flow rates applied to the different GC approaches.

3. Results and discussion

A sample of bergamot essential oil and a standard mixture of pesticides with different polarities and volatilities were analyzed by capillary GC under different speed approaches and analysis conditions (heating and flow rates) (see Table 2). The resulting separations were evaluated by the separation measure S as a descriptor of the metric of separation. Bergamot essential oil was chosen so as to evaluate how speed of analysis and conditions influence the separation parameters in a medium-to-low analysis temperature range, while the pesticide standard mixture was chosen for medium-to-high analysis temperatures. Figs. 1 and 2 show UFM-GC patterns of the bergamot essential oil and of the pesticide standard mixture analyzed with heating rates of 300 and 150 °C/min, respectively.

The first part of this study concerns the influence of GC approach (conventional GC, FGC10, FGC5, and UFM-GC) on separation parameters (S, t_b, S/t_b, n, and w), while the latter part deals with the influence of heating and flow rates on separation parameters when using UFM-GC. Columns of different lengths (5 and 10 m) were adopted for FGC to make the results comparable both to those obtained by conventional GC since the separation of a 10 m narrow-bore column is (or should be) comparable to that of a 25 m × 0.25 i.d. conventional column [6], provided that the same phase ratio is maintained, and to those of UFM-GC, which was run with a 5 m × 0.1 mm column.

3.1. Influence of different GC approaches on separation parameters

3.1.1. Bergamot essential oil

Table 3 reports S, t_b, S/t_b, n, and w calculated for bergamot essential oil when analyzed by conventional GC, FGC10, FGC5, and UFM-GC. S and n were measured in the time interval between α-pinene (t_a) and linalyl acetate (t_b); linalyl acetate retention time was also adopted as a measure of analysis time (t_b), being it the last eluting peak considered. All experiments were carried out in constant flow mode (Table 3).

The results show how the separation capability of the chromatographic system (i.e. S and n) and analysis time are conditioned by different GC approaches and heating rates. S and n decrease much less than the gain in analysis time: for instance, with bergamot essential oil, t_b with UFM-GC at 500 °C/min is 50 times shorter than that with conventional GC (1279.5 s

Table 2
GC approaches and heating and flow rates adopted in the present studies

GC approach	Heating rate (°C/min)									Flow (mL/min)					
	3	15	30	40	50	100	150	300	500	0.3	0.5	0.8	1	1.2	1.5
Conventional GC (25 m)	×														×
Fast GC (10 m)		×	×	×	×						×				
Fast GC (5 m)		×	×	×	×						×				
Ultra fast module-GC (5 m)						×	×	×	×	×	×	×	×	×	

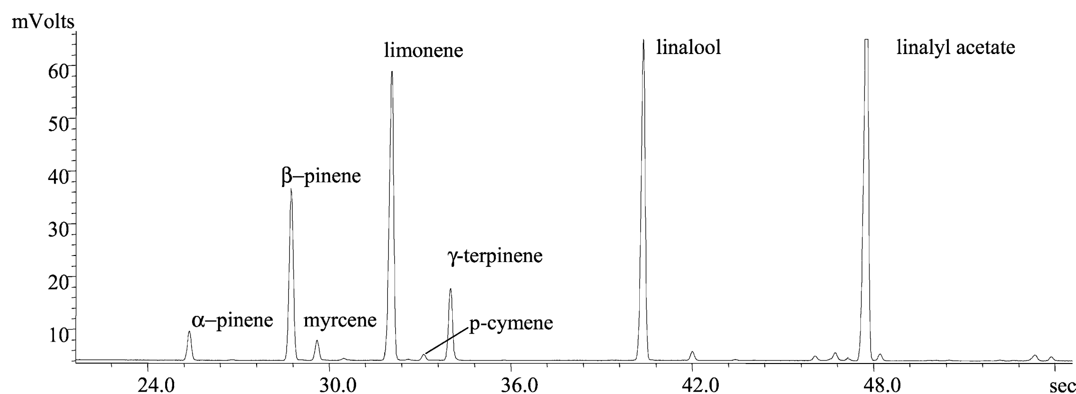

Fig. 1. UFM-GC pattern of the bergamot essential oil analyzed with a heating rate of 300 °C/min.

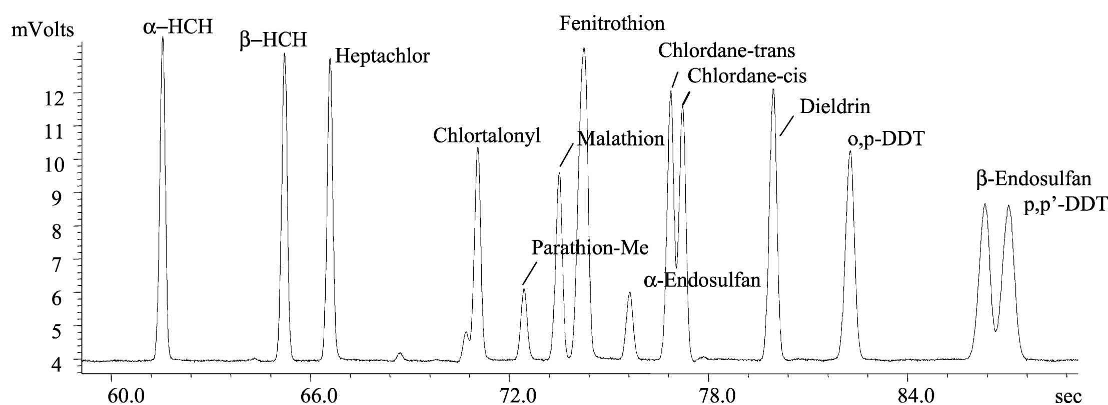

Fig. 2. UFM-GC pattern of the pesticide standard mixture analyzed with a heating rate of 150 °C/min.

(i.e. 21.3 min) and 25.6 s, respectively), while S and n of the chromatographic system is only five times lower than for conventional GC (S from 780 to 158, n from 135.5 to 27.1). Moreover, it is also evident how heating rate influences the separation capability of the chromatographic system: for instance, FGC5 at 30 °C/min gives S, n, and t_b very similar to those of FGC10 at 50 °C/min, while t_b with UFM-GC at 150 °C/min is about half that with FGC5 at 50 °C/min, and S and n are only reduced by about 30%.

Fig. 3 plots S (a) and S/t_b (b) versus heating rate in the time interval selected for bergamot essential oil analysis. As expected, S decreases when heating rate increases, but it drops

Table 3
Analysis times, peak widths, σ, S, and S/t_b for the bergamot essential oil determined after conventional GC, FGC with 5 and 10 m columns and UFM-GC analysis

	Conventional GC	FGC10				FGC5				UFM-GC			
Rate (°C/min)	3	15	30	40	50	15	30	40	50	100	150	300	500
t_o (s)	1.0	17.9	17.9	17.9	17.9	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
t_a (s) (α-pinene)	369.2	89.1	74.9	69.0	65.0	41.9	37.9	35.7	34.0	28.4	25.4	20.9	18.4
t_b (s) (linayl acetate)	1279.5	276.0	177.2	147.8	129.3	199.2	129.2	107.9	93.7	61.0	47.8	32.6	25.6
Width (a) at 50% (s)	2.12	0.48	0.36	0.32	0.28	0.38	0.30	0.27	0.25	0.18	0.15	0.11	0.09
Width (b) at 50% (s)	3.20	0.76	0.48	0.36	0.32	0.95	0.55	0.43	0.35	0.22	0.16	0.13	0.11
Peak capacity (n)	135.3	117.0	97.2	89.1	82.3	94.9	84.7	80.9	78.1	63.8	56.2	39.4	27.1
σ_a (s)	0.900	0.204	0.153	0.136	0.119	0.161	0.127	0.115	0.106	0.076	0.064	0.047	0.038
σ_b (s)	1.359	0.323	0.204	0.153	0.136	0.403	0.234	0.183	0.149	0.093	0.068	0.055	0.047
S_{ab}	780	668	572	524	482	525	480	464	453	376	333	236	158
S/t_b (s^{-1})	0.61	2.42	3.23	3.54	3.73	2.64	3.72	4.30	4.84	6.16	6.97	7.23	6.17
GC conditions	Flow: 1.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 50	Flow: 0.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 100				Flow: 0.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 150				Flow: 0.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 150			

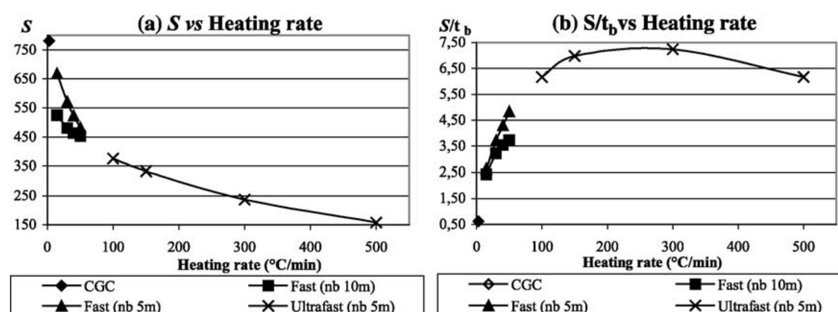

Fig. 3. S (a) and S/t_b (b) vs. heating rates for bergamot essential oil analysis.

less at higher heating rates (UFM-GC). When plotted versus heating rate, S/t_b achieves a maximum for UFM-GC at about 300 °C/min, which probably represents a good compromise between loss of separation capacity and reduction of analysis time. S/t_b behavior also confirms that analysis time decreases faster than the separation capability of the chromatographic system.

3.1.2. Pesticide standard mixture

Table 4 reports S, t_b, S/t_b, n, and w calculated for the analysis of the pesticide standard mixture when analyzed by conventional GC, FGC10, FGC5, and UFM-GC. S and n were measured in the time interval between α-HCH (t_a) and p,p'-DDT (t_b); p,p'-DDT retention time (t_b) was also adopted as a measure of analysis time (t_b), being the last eluting peak considered. All experiments were carried out in constant flow mode (Table 4). These results confirm those obtained with bergamot essential oil, showing that S, n, and t_b depend on the different GC approaches and heating rates; with the pesticide standard mixture, analysis time decreased from 1664 s (27.8 min) for conventional GC to 48.4 s with UFM-GC at 500 °C/min (a factor of about 35), while S decreased only from 627 to 181 (about 3.5) and n from 105.7 to 21.4 (about 5). Moreover, when column length is halved (FGC10 versus FGC5), if the same heating rate is used (50 °C/min), S, n, and t_b all decrease by about 20%; on the other hand, with columns of the same length (FGC5 and UFM-GC) heated at different rates (50 versus 150 °C/min), while t_b is less than halved (about 45%) S and n decrease only by about 30%.

Fig. 4 plots S (a) S/t_b (b) versus heating rate in the time interval selected for bergamot essential oil analysis. These diagrams confirm the results obtained with bergamot essential oil, since S drastically decreases when heating rate increases for conventional GC, FGC10, and FGC5, while for UFM-GC with heating rates above 150 °C/min, the drop in S tends to be small. Moreover, in UFM-GC with the same heating rates, S/t_b is likewise almost constant confirming that under constant flow and above a given heating rate (about 200 °C/min in this case), its influence is relatively small.

3.1.3. Influence of flow and heating rates on S, n, and analysis time, t_b, in UFM-GC

The second part of the study concerned the influence of flow and heating rates on the separation capability (S, n, S/t_b) and analysis time (t_b) when the two samples investigated are analyzed by UFM-GC. Table 2 summarizes the GC conditions applied. Linalyl acetate and p,p'-DDT retention times were again adopted as measures of analysis time (t_b), being the last eluting peaks considered for bergamot essential oil and for the standard mixture of pesticides, respectively.

Table 4
Analysis times, peak widths, σ, S, and S/t_b for the standard mixture of pesticides determined after conventional GC, FGC with 5 and 10 m columns and UFM-GC analysis

	Conventional GC	FGC10				FGC5				UFM-GC			
Rate (°C/min)	3	15	30	40	50	15	30	40	50	100	150	300	500
t_o (s)	57.6	17.0	17.0	17.0	17.0	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
t_a (s) (α-HCH)	1002	332.2	210.9	175.4	153.0	248.9	160.9	133.7	117.2	77.3	61.4	43.0	34.8
t_b (s) (p,p'-DDT)	1664	560.8	330.0	266.8	227.4	473.6	274.7	220.4	185.9	112.8	86.0	58.8	48.4
Width (a) at 50% (s)	2.37	0.80	0.48	0.40	0.36	1.02	0.59	0.47	0.41	0.23	0.18	0.12	0.10
Width (b) at 50% (s)	2.40	0.88	0.56	0.40	0.44	1.42	0.71	0.56	0.48	0.33	0.33	0.40	0.33
Peak capacity	105.7	103.6	87.7	82.8	73.1	71.7	66.0	63.4	61.3	45.0	34.6	24.1	21.4
σ_a (s)	1.006	0.340	0.204	0.170	0.153	0.433	0.251	0.200	0.174	0.098	0.076	0.051	0.042
σ_b (s)	1.019	0.374	0.238	0.170	0.187	0.603	0.301	0.238	0.204	0.140	0.140	0.170	0.140
S_{ab}	627	619	530	478	451	446	397	381	378	320	269	197	181
S/t_b (s^{-1})	0.38	1.10	1.61	1.79	1.98	0.94	1.44	1.73	2.03	2.83	3.12	3.35	3.73
GC conditions	Flow: 1.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 50	Flow: 0.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 100				Flow: 0.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 150				Flow: 0.5 mL/min; T_{inj}: 250 °C; T_{det}: 270 °C; SR: 150			

S was measured in the time interval between α-HCH and p,p'-DDT.

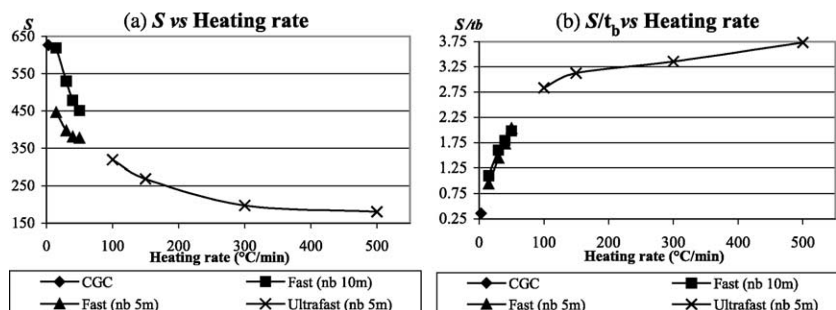

Fig. 4. S (a) and S/t_b (b) vs. heating rates for the pesticide standard mixture analysis.

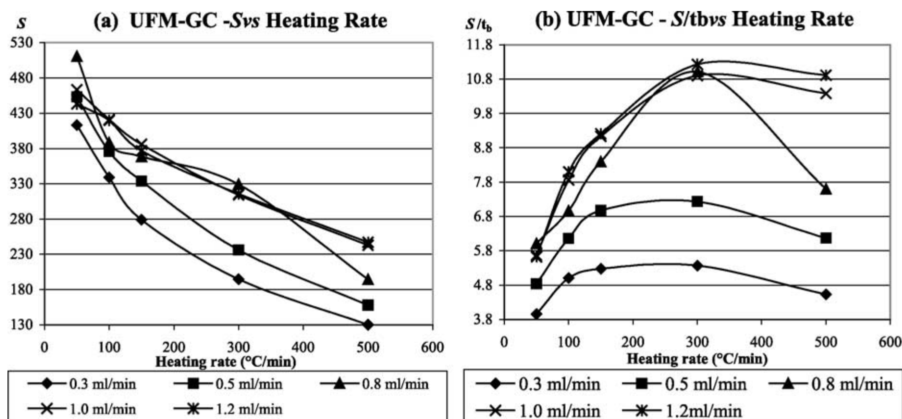

Fig. 5. S (a) and S/t_b (b) vs. heating rates for bergamot essential oil analysis.

3.1.4. Bergamot essential oil

Table 5 shows how S, t_b, n, and S/t_b vary when bergamot essential oil is analyzed by UFM-GC with different flow and heating rates. S-values were measured in the time interval between the elution of α-pinene (a) and limonene (c) (S_{ac}), limonene (c) and linalyl acetate (b) (S_{cb}) and α-pinene (a) and linalyl acetate (b) (S_{ab}). Fig. 5 plots S (a) and S/t_b (b) versus heating rates and Fig. 6 plots S (a) and S/t_b (b) versus flow rates in the time intervals selected for bergamot essential oil UFM-GC analysis.

These results show how S and t_b are conditioned by different flow and heating rates. As expected, in UFM-GC, an increase in heating rate induces a decrease of S, which is lower when flow rates above 0.8 mL/min are used (Fig. 4a). Moreover, for each heating rate, an optimal flow rate can be found to maximize the separation capability of the

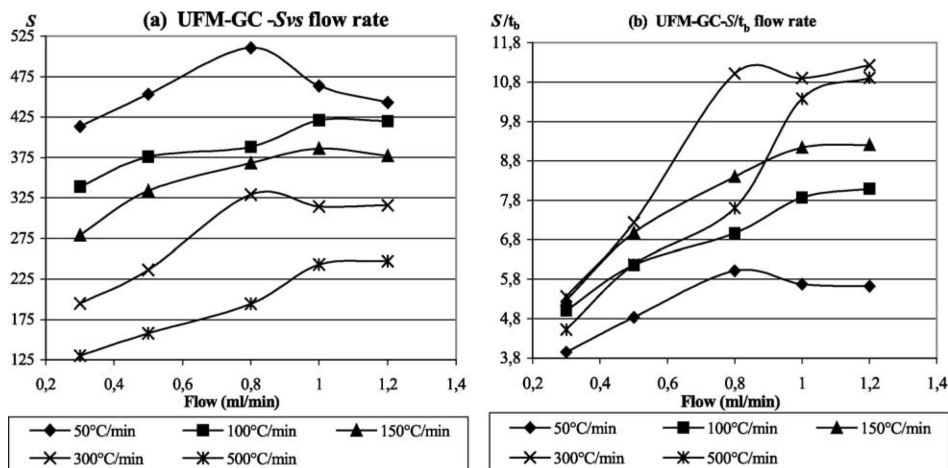

Fig. 6. S vs. flow rates (a) and S/t_b vs. flow rates (b) for bergamot essential oil analysis.

Table 5

Influence of flow and heating rates on t_b, S, n, and S/t_b for bergamot essential oil when analyzed by UFM-GC

GC parameters	UFM-GC conditions																								
Rate (°C/min)	50					100					150					300					500				
Flow (mL/min)	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2
t_o	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7
t_a (α-pinene)	42.8	34.0	27.3	24.7	22.7	35.0	28.4	23.8	21.6	20.2	30.8	25.4	21.7	19.8	18.5	25.0	20.9	18.0	16.8	16.0	21.9	18.4	18.4	16.0	15.1
t_c (limonene)	59.6	49.6	41.6	38.6	36.1	44.6	37.8	32.6	30.4	28.9	37.4	32.1	28.1	26.3	25.0	28.5	24.6	21.8	20.6	19.8	24.0	20.7	20.7	18.4	17.6
t_b (linayl acetate)	104.5	93.7	84.9	81.7	78.8	67.6	61.0	55.7	53.5	51.8	52.9	47.8	43.9	42.2	40.9	36.2	32.6	29.9	28.8	28.1	28.7	25.6	25.6	23.4	22.6
Width at 50% (a)	0.31	0.25	0.21	0.21	0.21	0.23	0.18	0.19	0.17	0.15	0.19	0.15	0.13	0.12	0.13	0.14	0.11	0.09	0.09	0.09	0.13	0.09	0.09	0.08	0.08
Width at 50% (c)	0.35	0.3	0.25	0.27	0.28	0.23	0.2	0.19	0.18	0.17	0.19	0.16	0.14	0.14	0.13	0.14	0.11	0.08	0.09	0.09	0.13	0.11	0.09	0.07	0.07
Width at 50% (b)	0.37	0.35	0.31	0.35	0.36	0.22	0.22	0.2	0.18	0.2	0.18	0.16	0.15	0.14	0.16	0.13	0.13	0.09	0.09	0.09	0.11	0.11	0.08	0.07	0.07
Peak capacity (n)	70.4	78.1	88.1	80.8	77.7	56.4	63.8	64.8	70.9	71.6	46.4	56.2	62.2	66.1	62.9	32.4	39.4	53.8	52.3	52.6	21.6	27.1	32.3	39.5	40.1
σ_a	0.132	0.106	0.089	0.089	0.089	0.098	0.076	0.081	0.072	0.064	0.081	0.064	0.055	0.051	0.055	0.059	0.047	0.038	0.038	0.038	0.055	0.038	0.038	0.034	0.034
σ_c	0.149	0.127	0.106	0.115	0.119	0.098	0.085	0.081	0.076	0.072	0.081	0.068	0.059	0.059	0.055	0.059	0.047	0.034	0.038	0.038	0.055	0.047	0.038	0.030	0.030
σ_b	0.157	0.149	0.132	0.149	0.153	0.093	0.093	0.085	0.076	0.085	0.076	0.068	0.064	0.059	0.068	0.055	0.055	0.038	0.038	0.038	0.047	0.047	0.034	0.030	0.030
S_{ac}	120	134	146	136	130	98.3	116	109	118	127	82.5	102	112	118	118	59.5	78.4	105	98.9	99.2	38.0	53.7	59.7	75.4	77.2
S_{cb}	293	320	364	327	314	241	260	279	303	293	196	231	256	267	258	135	158	224	215	216	91.8	104	134	167	170
S_{ab}	413	453	511	463	443	339	376	388	421	420	279	333	368	386	377	195	236	329	314	316	130	158	194	243	247
S/t_b	3.95	4.84	6.01	5.67	5.63	5.01	6.16	6.97	7.86	8.10	5.28	6.97	8.40	9.14	9.21	5.37	7.23	11.02	10.90	11.23	4.53	6.17	7.60	10.38	10.91

Table 6

Influence of flow and heating rates on t_b, S, n, and S/t_b for pesticide standard mixture when analyzed by UFM-GC

GC parameters	UFM-GC conditions																								
Rate (°C/min)	50					100					150					300					500				
Flow (mL/min)	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2	0.3	0.5	0.8	1	1.2
t_o	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7	9.4	6.6	4.8	4.2	3.7
t_a (α-HCH)	130.1	117.6	108.5	104.2	101.2	84.8	77.6	71.8	69.4	67.6	67.2	61.6	57.3	55.5	54.1	47.1	43.1	40.3	39.1	38.3	38.4	34.8	32.4	31.4	30.8
t_c (dieldrin)	181.9	169.1	159.0	154.1	150.8	111.7	104.0	97.9	95.6	93.5	86.5	80.0	75.3	73.3	71.7	60.8	54.4	50.4	48.9	47.8	50.8	44.2	40.1	38.6	37.6
t_b (p,p'-DDT)	200.4	186.8	177.4	173.0	169.9	121.7	113.5	107.1	104.7	102.5	95.4	87.1	81.3	79.7	78.1	68.9	60.6	55.3	53.3	51.9	59.1	50.2	44.7	42.6	41.2
Width at 50% (a)	0.48	0.39	0.37	0.38	0.39	0.30	0.24	0.22	0.23	0.22	0.23	0.19	0.17	0.17	0.17	0.17	0.12	0.11	0.11	0.11	0.17	0.12	0.08	0.09	0.09
Width at 50% (c)	0.56	0.46	0.48	0.46	0.51	0.39	0.30	0.27	0.26	0.25	0.38	0.26	0.23	0.22	0.22	0.41	0.27	0.21	0.19	0.17	0.44	0.28	0.21	0.20	0.17
Width at 50% (b)	0.69	0.49	0.50	0.51	0.58	0.55	0.40	0.34	0.35	0.34	0.65	0.42	0.35	0.32	0.31	0.63	0.46	0.39	0.38	0.35	0.61	0.45	0.38	0.34	0.28
Peak capacity (n)	47.9	60.8	60.1	59.9	54.7	35.1	45.0	50.1	49.4	50.7	26.3	34.6	37.6	40.2	40.3	21.2	24.1	24.8	24.5	25.3	19.9	21.3	21.6	20.9	22.7
σ_a	0.204	0.166	0.157	0.161	0.166	0.127	0.102	0.093	0.098	0.093	0.098	0.081	0.072	0.072	0.072	0.072	0.051	0.047	0.047	0.047	0.072	0.051	0.034	0.038	0.038
σ_c	0.238	0.195	0.204	0.195	0.217	0.166	0.127	0.115	0.110	0.106	0.161	0.110	0.098	0.093	0.093	0.174	0.115	0.089	0.081	0.072	0.187	0.119	0.089	0.085	0.072
σ_b	0.293	0.208	0.212	0.217	0.246	0.234	0.170	0.144	0.149	0.144	0.276	0.178	0.149	0.136	0.132	0.268	0.195	0.166	0.161	0.149	0.259	0.191	0.161	0.144	0.119
S_{ac}	234	285	280	279	260	184	231	251	252	259	149	193	211	215	212	111	136	148	153	159	95.6	111	125	116	123
S_{cb}	69.9	88.0	88.4	91.8	82.5	50.2	63.9	71.0	70.3	71.8	40.7	49.2	48.7	56.3	56.4	36.9	39.7	38.1	36.4	36.7	37.0	38.7	36.3	34.9	37.7
S_{ab}	304	373	368	371	342	234	294	322	322	331	190	242	260	271	269	148	176	186	190	196	133	150	162	151	161
S/t_{ab}	1.52	2.00	2.08	2.15	2.01	1.92	2.59	3.01	3.08	3.23	1.99	2.78	3.20	3.40	3.45	2.15	2.91	3.37	3.56	3.79	2.25	2.98	3.62	3.55	3.91

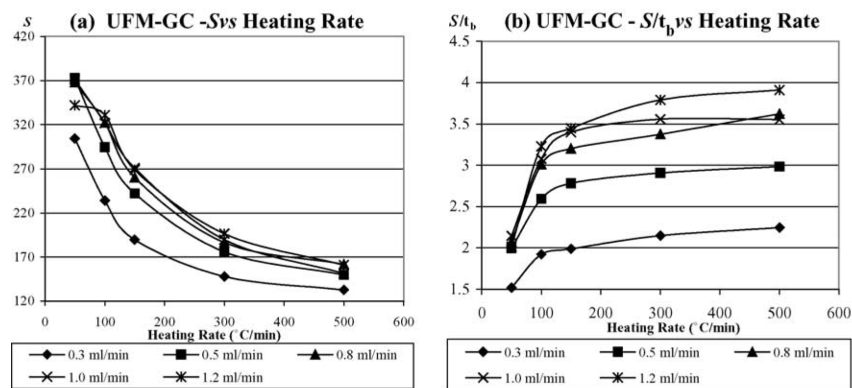

Fig. 7. S (a) and S/t_b (b) vs. heating rates for pesticide standard mixture analysis.

chromatographic system (Fig. 5a). On the other hand, the best separation capability in the shortest analysis time (S/t_b) is achieved at optimal heating and flow rates (Figs. 4b and 5b), that for bergamot essential oil are 300 °C/min and above 0.8 mL/min, respectively.

3.1.5. Pesticide standard mixture

Table 6 shows how S, t_b, n, and S/t_b vary when the pesticide standard mixture is analyzed by UFM-GC under different flow and heating rates. S-values were measured in the time interval between the elution of α-HCH (a) and dieldrin (c) (S_{ac}), dieldrin (c) and p,p'-DDT (b) (S_{cb}) and α-HCH (a) and p,p'-DDT (b) (S_{ab}). Fig. 7 plots S_{ab} values (a) and S_{ab}/t_b (b) versus heating rates and Fig. 8 plots S_{ab} (a) and S_{ab}/t_b (b) versus flow rates for time intervals selected in the pesticide standard mixture UFM-GC analysis.

These results confirm that also with the pesticide standard mixture S and t_b are conditioned by flow and heating rates. In this case too, an increase in heating rate produces a decrease in S, although to a lesser extent than with bergamot essential oil when flow rates is above 0.5 mL/min (Fig. 7a). Moreover, with the sample investigated and within each heating rate, S variation with flow rates was less pronounced than with the bergamot essential oil and comparable S-values were obtained between 0.5 and 1.2 mL/min (Fig. 8a). Similar results are obtained with S/t_b: with the same heating rate, the separation capability over time was quite similar with flow rate above 0.8 mL/min, while with the same flow rate, similar separation was obtained above 150 °C/min. The different behavior of the two samples is probably due to the lower volatility of the pesticides investigated compared to the components of bergamot essential oil.

3.1.6. S versus R of critical pairs of analytes

The results reported in the above sections enable to determine the separation capability of a chromatographic system required when critical couples of analytes in a sample must be resolved. All considerations are only valid within each GC approach, because peak widths (i.e. non-overlapping σ) differ for each of them and decrease when the speed of the GC analysis increases; as a consequence, a faster GC approach requires lower S-values to achieve the same separation. An illustrative example is the separation of the β-endosulfan/p,p'-DDT pair in the pesticide standard mixture investigated, when the two analytes must be base line separated in the shortest time. In this case too, S was measured in the time interval

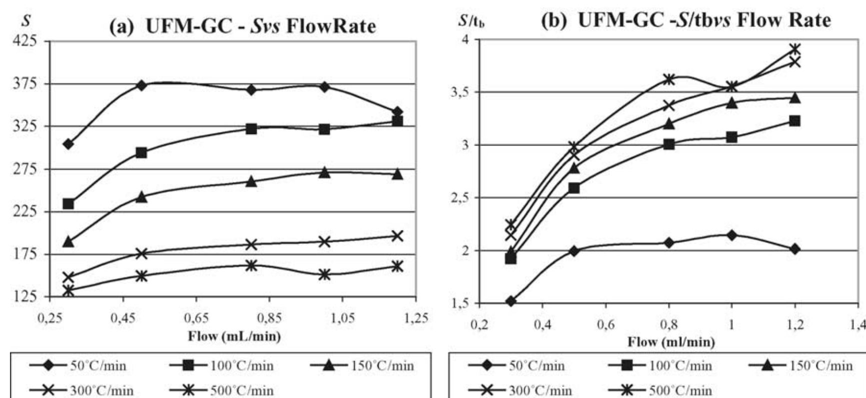

Fig. 8. S (a) and S/t_b (b) vs. flow rates for pesticide standard mixture analysis.

Table 7
Heating rate, flow rate, analysis time, S and resolution of the β-endosulfan/p,p'-DDT pair when analyzed by conventional GC, FGC10, FGC5, and UFM-GC analysis

	Conventional GC	FGC10	FGC5	UFM-GC		
Heating rate (°C/min)	3	40	50	150	150	300
Flow rate (mL/min)	1.5	0.5	0.5	0.5	1	1.2
t_b (s) (p,p'-DDT)	1664.0	266.8	185.9	87.1	79.7	51.9
S_{ab}	627	478	378	242	271	196
Resolution (R)	5.9	1.8	2.6	0.95	1.5	0.7

S was measured in the time interval between α-HCH and p,p'-DDT.

between α-HCH and p,p'-DDT and the retention time of p,p'-DDT was taken as analysis time since it is the last eluting peak in the mixture. Fig. 9 reports the separation of β-endosulfan and p,p'-DDT when analyzed by conventional GC (a), by FGC10 (b), by FGC5 (c), by UFM-GC at 150 °C/min, and 0.5 mL/min (d), UFM-GC at 150 °C/min and 1 mL/min (e) and by UFM-GC at 300 °C/min and 1.2 mL/min (f). Table 7 reports heating rate, flow rate, analysis time, S and resolution of the endosulfan/p,p'-DDT pair when analyzed by conventional GC, FGC10, FGC5, and UFM-GC analysis. Under the conditions applied, in conventional GC S-value is 627 and the two peaks are very well separated with an R of 5.9, although an analysis time of 1664 s (27.7 min) is necessary. In FGC10 the base line separation of the pair in question requires an S-value of at least 478 to obtain an R of 1.8 and the analysis time drastically decreases to 267 s (4.5 min), while for FGC5 an S-value of 378 implies a resolution of 2.6 and an analysis time of 186 s (3.1 min). In UFM-GC, the β-endosulfan/p,p'-DDT pair is not base line separated at 150 °C/min with a flow rate of 0.5 mL/min ($S = 242$, $R = 0.95$): flows adequate (above 1 mL/min) to increase S to at least 270 are needed in order to obtain a resolution of 1.5 reducing the analysis time to 80 s. Moreover, in UFM-GC a base line separation of this pair could not be achieved at heating rates above 150 °C/min whichever is flow, without using a different stationary phase. From these results it is clear that within a specific GC approach, the chromatographic system (stationary phase, heating and flow rates) must achieve a minimum S-value to be able to separate two analytes with a given resolution in a minimum analysis time.

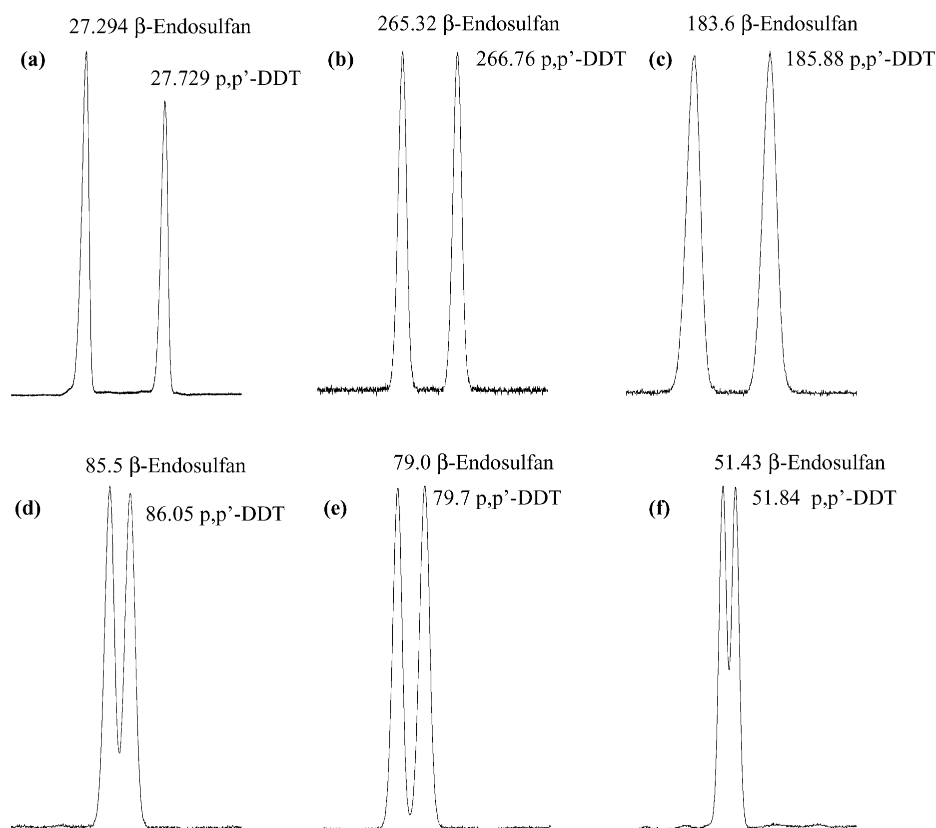

Fig. 9. Separations of β-endosulfan and p,p'-DDT when analyzed by CGC (a), FGC10 (b), FGC5 (c), UFM-GC at 150 °C/min and 0.5 mL/min (d), UFM-GC at 150 °C/min and 1 mL/min (e), and UFM-GC at 300 °C/min and 1.2 mL/min (f).

4. Conclusions

The results of this study show that the separation measure S can successfully be used to evaluate the separation capability of chromatographic systems when real world samples are analyzed with different GC approaches. In particular, these results show that in constant flow mode the heating rate conditions separation capability (i.e. S and n) of the GC system and analysis time of a sample, but S and n decrease much less than the gain in analysis time in particular when high heating rates are applied. Flow rate also plays an important role: the experiments carried out in UFM-GC show that an appropriate flow choice can partly compensate the loss of separation capability due to the heating rate increase and contribute to increase S and to reduce t_b. Last but not least, within a specific GC approach, to separate two analytes with a given resolution while minimizing analysis time, the chromatographic system (column characteristic and stationary phase) and conditions (flow and heating rates) must be such as to achieve a high enough S-value.

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