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Conventional inner diameter short capillary columns: an approach to speeding up gas chromatographic analysis of medium complexity samples

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Abstract

Short capillary columns (5 m) with 0.25 mm inner diameter (I.D.) are applied to the GC analysis of medium complexity samples (up to 30 components) with the aim of shortening analysis time. This approach is complementary to fast GC with narrow-bore columns and is based on compensating the lower efficiency of short columns with conventional I.D.'s (0.25–0.32 mm) by using a stationary phase selectivity suitable to separate the components of the sample under investigation, so that the required resolution power is achieved but, at the same time, the analysis time is shortened. The qualitative and quantitative effectiveness of this approach is demonstrated through the analysis of: essential oils with different compositions (chamomile and rosemary), low-volatility triterpenes in a plant extract (*Maytenus aquifolium* and *M. ilicfolium*), thermolabile pyrethrins in a Pyrethrum extract, and a mixture of pesticides applied to protect medicinal plant crops. In all examples, GC analysis was five to ten times faster than with conventional columns.

Keywords: Fast analysis; Short columns; Column diameter; Essential oils; Triterpenes; Pyrethrins

1. Introduction

There is a continual demand for faster GC separation. Although investigated since the early 60s [1–3], only over the last decade, have some approaches been proposed and applied to routine analysis. The various approaches for fast GC have recently been reviewed and discussed in theoretical terms by Cramers et al. [4].

The easiest way to speed up a GC separation is to decrease column length while keeping enough resolving power for the given separation problem. Short columns with narrow inner diameters (I.D.) (0.1 mm or less) offer efficiency and peak capacity comparable to those of columns with conventional length and I.D. (0.25–0.32 mm I.D.) and can be used when high resolution is needed to analyse a complex mixture correctly. This is clear from Eq. (1), which

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leaves out of consideration extra-column band broadening and gas compression [5]:

$$\text{HETP} = \frac{f(k) \cdot r^2 \cdot u}{D} \quad (1)$$

where HETP is the height equivalent to a theoretical plate; $f(k)$ is a function depending on the retention factor, which, in its turn, depends on the stationary phase, phase ratios and column temperature; r is the column radius; u is the average linear velocity of the mobile phase; and D is the diffusion coefficient. But, since

$$\text{HETP} = \frac{L}{n}$$

where L is the column length and n the theoretical plate number, Eq. (1) can be rewritten:

$$n = \frac{D \cdot L}{f(k) \cdot r^2 \cdot u} \quad (2)$$

Eq. (2) shows that when column length is shortened, column efficiency (n) can be kept constant by reducing column radius and/or the average linear velocity of the carrier gas. Routine experience has shown that, when the length/I.D. ratio is unvaried, a short narrow bore column has the same efficiency as a conventional column coated with the same stationary phase, i.e. a 10 m×0.10 mm I.D. narrow bore column has the same resolving power as a 25 m×0.25 mm I.D. column [6]. With short columns, analysis time is also shortened because higher average gas velocities can be used without losing efficiency, since the gas-compression effect is lower and thus the Van Deemter curves (H/u plots) of the carrier gas are flatter [7].

Narrow-bore capillary columns (mainly 0.10 mm I.D.) are the most popular approach nowadays adopted for high-speed GC (HSGC) in routine analysis, but they require to be assembled in recent instrumentation provided with electronic pressure control and high frequency FID detectors to exploit their capability thoroughly. Quality control laboratories also need software suitable to avoid method re-validation being necessary when conventional I.D. columns are replaced by narrow bore columns [6].

The most critical points of narrow bore columns are their low sample loading (in the nl range) requiring very high split ratios, which can cause irregular sample transfer into the column and irreproducibility with samples containing analytes at widely differing concentration [8], and the fast peak elution from the column requiring fast detectors and MS-systems (such as time-of-flight MS) in particular when reliable quantitative detection is necessary [4]. Moreover, narrow-bore columns are not compatible with cold-on-column injection, thus they cannot be used to analyse thermolabile compounds.

It is well known that for a large number of applications the efficiency of a capillary column is much higher than necessary. GC separation depends on column efficiency and on stationary phase selectivity. The separation power of capillary columns is mainly based on efficiency, which is so high that selectivity may often be ‘‘neglected’’ because most separations are achieved without the selectivity of the stationary phase playing a crucial role. On the contrary, selectivity was the fundamental parameter for packed columns because of their very low efficiency. This means that the minimal number of theoretical plates required to analyse a given complexity mixture (n_{req}) can also be achieved with the concurrence of a suitable stationary phase. These considerations provide the basis for an approach, different from narrow bore columns, to speeding up GC analysis for medium complexity samples where the efficiency of shorter columns with conventional I.D.’s (0.25–0.32 mm) is combined with a selectivity suitable to separate the components in the sample under investigation, so that the resolution power of the chromatographic system is maintained. In this way, the loss of efficiency due to reduction in column length (i.e. 20–50 000 theoretical plates instead of the usual 150 000 of a conventional 25 m capillary column) is compensated by better selectivity. The loss of efficiency of short columns with conventional I.D. is lower than expected because incidentally, they operate at lower inlet pressure and this gives a higher diffusion coefficient (D) (see Eq. (2)).

This study aims to evaluate how effective conventional I.D. short columns can be in speeding up GC analyses, by looking at some applications to medium complexity real-world samples.

2. Experimental

Rosemary and chamomile essential oils (e.o.) were obtained by hydrodistillation by the method described in the European Pharmacopoeia [9]. *Maytenus aquifolium* and *M. ilicfolium* extracts were prepared by sonicating plant material in *n*-hexane and then cleaning-up the crude extract by SPE on a Florisil cartridge with chloroform [10]. Pyrethrum extract was obtained by extracting dried plant material with petroleum–ether in a Soxhlet apparatus for 4 h and re-extracting with acetonitrile and evaporated to dryness.

2.1. GC analysis

GC analyses were carried out on a ThermoQuest Trace GC unit provided with flame ionization detection (FID) (Rodano – Italy). Data processing was by Chrom-card software (ThermoQuest Rodano – Italy) (Version 1.06 -32 bit). A series of fused-silica open tubular (FSOT)-high temperature silylated columns of different length were used. All columns were from MEGA (Legnano – Italy). Table 1 reports the list and the characteristics of the columns used in the present study.

For simplicity's sake, 25 m columns will be referred to as ''*conventional columns*'' and 5 m columns as ''*short columns*''; the inner diameter of all columns used in this study is 0.25 mm. Unless not specified otherwise, all results reported in the tables are the average percentages of the investigated components calculated over three analyses.

2.2. Rosemary essential oil

A 1 μl sample of a solution prepared by diluting 5 mg of rosemary e.o. in 1 ml of cyclohexane (1:200) was injected into the GC system under the following conditions:

Conventional column: SE-54 (column 5); injection: split, split ratio: 1:20, temperature: 230°C; detector: FID, temperature: 250°C; temperature programme: from 50°C (1 min) to 170°C at 3°C/min then to 220°C (1 min) at 30°C/min. Carrier gas: hydrogen, const. press.: 65 kPa.

Short column: Carbowax (CW) 20M (column 10); injection: split, split ratio: 1:20, temperature: 230°C; detector: FID, temperature: 250°C; temperature programme: from 50°C (1 min) to 80°C at 40°C/min then 130°C at 20°C/min and to 200°C at 75°C/min. Carrier gas: hydrogen, pressure programme: from 10 kPa (2 min) to 34 kPa at 200 kPa/min until to the end of the analysis.

2.3. Chamomile essential oil

A 1 μl sample of a solution prepared by diluting 5 mg of chamomile e.o. in 1 ml of cyclohexane (1:200) was injected into the GC system under the following conditions:

Conventional column: SE-54 (column 5); injection: split, split ratio: 1:20, temperature: 230°C; detector: FID, temperature: 250°C; temperature programme: from 50°C (1 min) to 190°C at 3°C/min then to 220°C at 20°C/min. Carrier gas: hydrogen, const. pressure: 65 kPa.

Short column: OV-1701 (column 8); injection:

Table 1
List and characteristics of the columns used for the present study

Stationary phases	Acronym	Column characteristics					
		I.D. 0.25 mm	Df μm	No.	25 m	No.	5 m
Polydimethylsiloxane	OV-1	X	0.3	1	X	2	X
Polydimethylsiloxane, 5% phenyl–	SE-52	X	0.25	3	X	4	X
Polydimethylsiloxane, 5% phenyl–, 5% vinyl	SE-54	X	0.25	5	X	6	X
Polydimethylsiloxane, 5% phenyl–, 7% cyanopropyl–	OV-1701	X	0.3	7	X	8	X
Polyethyleneglycol	CW-20M	X	0.5	9	X	10	X

split, split ratio: 1:20, temperature: 230°C; detector: FID, temperature: 280°C; temperature programme: from 100 to 250°C (2 min) at 40°C/min. Carrier gas: hydrogen, const. flow: 1.4 ml/min.

2.4. Maytenus aquifolium extracts

A 1 μl sample of a solution prepared by dissolving 10 mg of *M. aquifolium* extract in 1 ml of chloroform (1:100) was injected into the GC system.

Conventional column: SE-52 (column 3); injection: split, split ratio: 1:20, temperature: 280°C; detector: FID, temperature: 300°C; temperature programme: from 150°C (1 min) to 290°C (20 min) at 5°C/min. Carrier gas: hydrogen, const. pressure: 60 kPa.

Short column: SE-54 (column 4); injection: split, split ratio: 1:20, temperature: 250°C; detector: FID, temperature: 280°C; temperature programme: from 165 to 225°C at 30°C/min, then to 240°C at 5°C/min and to 280°C (1 min) at 40°C/min; carrier gas: hydrogen, const. flow: 2.0 ml/min.

2.5. Pyrethrum extract

A 1 μl sample of a solution prepared by dissolving 5 mg in 1 ml of cyclohexane (1:200) of pyrethrum extract was injected into the GC system. A standard solution of pyrethrins from a technical standard mixture (Pestanal®, Riedel-de-Haën, Milan (Italy)) was also used.

Conventional column: SE-54 (column 5); injection: split, split ratio: 1:20, temperature: 250°C; detector: FID, temperature: 280°C; temperature programme: from 50°C (1 min) to 190°C at 3°C/min then to 220°C at 20°C/min. Carrier gas: hydrogen, const. pressure: 60 kPa.

Short column: OV-1 (column 2); detector: FID, temperature: 280°C; carrier gas: hydrogen, const. flow: 5.0 ml/min.

(a) injection: split, split ratio: 1:20, temperature: 250°C; temperature programme: from 50 to 180°C at 50°C/min then to 200°C at 10°C/min and to 250°C at 30°C/min;

(b) injection: cold-on-column; temperature programme: from 50 to 180°C at 50°C/min then to 200°C at 10°C/min and to 250°C (2 min) at 30°C/min. With cold-on-column injection, the short OV-l column was connected to 5 m uncoated retention gap.

2.6. Organo-chlorine and organo-phosphorous pesticides

Pesticides and their concentrations in the standard solution are reported in Table 5. A 1 μl sample of a standard solution prepared by dissolving different amounts of organo-chlorine and organo-phosphorous pesticides in 1 ml of *n*-hexane was injected into the GC system.

Conventional columns: SE-52 (column 3); injection: split, split ratio: 1:20, temperature: 280°C; detector: FID, temperature: 300°C; temperature programme: from 140°C (1 min) to 270°C (3 min) at 10°C/mm; carrier gas: hydrogen, const. pressure: 60 kPa.

OV-1701 (column 7); injection: split, split ratio: 1:20, temperature: 280°C; detector: FID, temperature: 300°C; temperature programme: from 100°C (1 min) to 270°C (5 min) at 10°C/min; carrier gas: hydrogen, flow-rate: 1.4 ml/min.

Short column: OV-1701 (column 8); injection: split, split ratio: 1:20, temperature: 280°C; detector: FID, temperature: 300°C; temperature programme: from 140 to 270°C at 65°C/min (2 min); carrier gas: hydrogen, press. programme: from 10 kPa (1.9 min) to 50 kPa at 40 kPa/min (1.3 min).

3. Results and discussion

Short columns can be applied to routine quantitative analysis of medium complexity mixture (up to 30 components) or of a selected number of analytes in a complex sample provided that they are properly (baseline) separated through a stationary phase of suitable selectivity. Unlike conventional columns where a single column with high efficiency compensates for any lack of selectivity, this approach requires a set of short columns coated with different polarity stationary phases covering as wide as possible a range of selectivity. Table 1 reports the stationary phases and characteristics of the columns used in the present study. Similarly to fast GC with narrow-bore columns, GC analysis with short columns requires GC instruments provided with elec-

tronic pressure control of the carrier gas to optimise separation, ovens whose temperature can be programmed to provide fast heating rates and high frequency digital output detection [11]. Although reduction of analysis time is mainly due to the short length of the column, carrier gas electronic pressure control is often helpful not only in speeding-up an analysis but also in achieving a suitable efficiency to separate critical pairs of analytes (see rosemary essential oil, Pyrethrum extract and pesticide standard mixture analyses below) [11]. On the other hand, the usual split ratio can be applied since short columns have the usual sample capacity, thus avoiding irregular sample transfer and split irreproducibility with samples containing analytes at widely differing concentrations, and thermolabile analytes can be analysed by cold-on-column injection provided that the column is connected to a suitable retention gap.

Short columns are here applied to samples containing analytes with different polarities, structures and volatilities, and the results are compared to those of conventional columns.

Rosemary (*Rosmarinus officinalis* L.) essential oil (e.o.) is widely used as food flavouring, in aromatherapy and in traditional medicine. Rosemary e.o. is a medium complexity e.o. that is commercially characterised by medium-to-high volatility components, in particular α-pinene, camphene, β-pinene, limonene, 1,8-cineole, camphor, linalool, borneol and bornyl acetate [12]. These components are generally baseline separated with conventional apolar columns such as OV-1, SE-52 or SE-54 in about 20 min; the corresponding short columns (e.g. SE-52) were not efficient and/or selective enough to separate the most volatile components, for instance the limonene/1,8-cineol pairs. Suitable selectivity for this separation was obtained with more polar stationary phases: the characteristic components are all separated with OV-1701 but in about 10 min; analysis time could be further reduced to less than 4 min, while keeping a resolving power suitable for this separation, with a more polar stationary phase such as CW 20M. The analysis time with a conventional CW 20M column was about 25 min. A carrier gas pressure programme not only speeded up analysis but also separated the limonene/1,8-cineol and

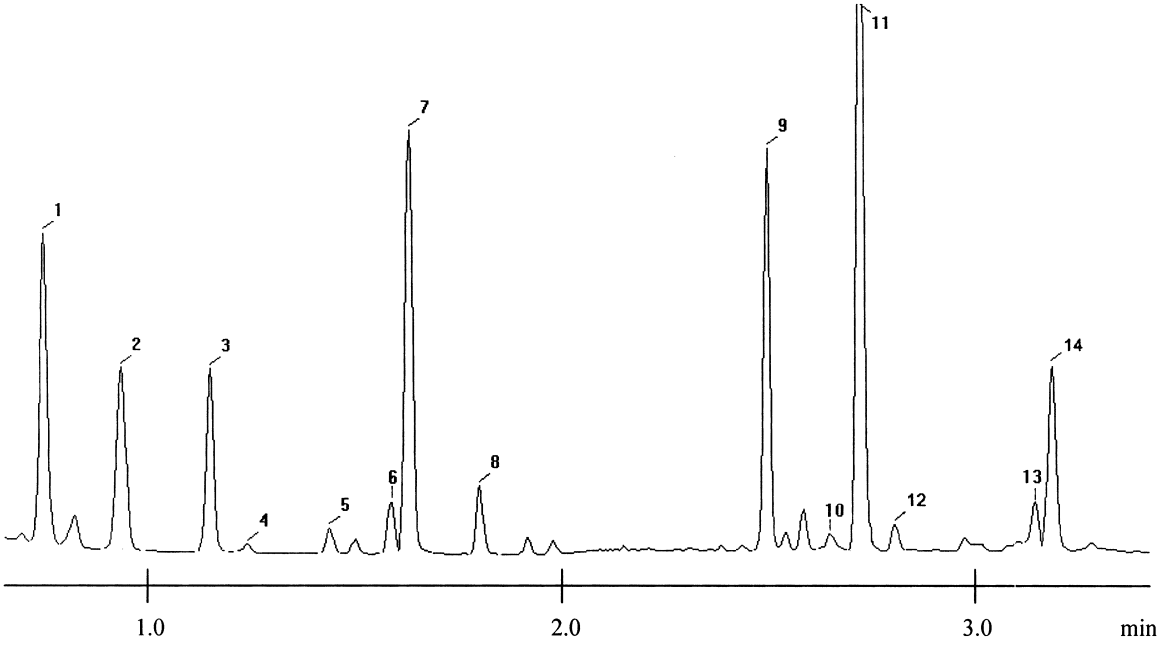

Fig. 1. cGC pattern of a rosemary oil analysed with a short CW 20M column. 1: α-pinene, 2: camphene, 3: β-pinene, 4: myrcene, 5: α-phellandrene, 6: limonene, 7: 1,8-cineole, 8: γ-terpinene, 9: camphor, 10: linalool, 11: bornyl acetate, 12: α-terpineol, 13: verbenone, 14: borneol.

Table 2
Retention times (t_R) and average percentages of the characteristic components of rosemary e.o. calculated over three analyses

No.	Compounds	Columns			
		CW 20M 25 m		CW 20M 5 m	
		t_R (min)	Area %	t_R (min)	Area %
1	α-Pinene	2.72	9.4	0.75	10.6
2	Camphene	3.27	9.0	0.94	9.0
3	β-Pinene	3.92	6.8	1.16	7.3
4	Mycene	4.25	0.2	1.26	0.4
5	α-Phellandrene	5.14	0.4	1.46	1.0
6	Limonene	5.89	1.6	1.61	1.9
7	1,8-Cineole	6.11	13.7	1.66	14.4
8	γ-Terpinene	7.19	1.9	1.83	2.4
9	Camphor	16.22	14.0	2.55	13.4
10	Linalool	18.49	0.5	2.70	0.9
11	Bornyl acetate	19.16	30.8	2.78	28.6
12	α-Terpineol	20.12	1.0	2.87	0.9
13	Verbenone	23.41	1.7	3.22	1.8
14	Borneol	23.91	8.2	3.26	7.7

verbenone/bornyl acetate pairs. Fig. 1 reports the cGC pattern of a rosemary oil analysed with a short CW 20M column, while Table 2 reports the average percentages of the components of interest calculated over three analyses with the conventional SE-52 and short CW 20M columns. These results show that correlation between the data obtained with the two columns is very good. In this example, the separation is tuned on a small number of characterising components and the analysis time with a short column is more than five times less than with a conventional column.

Chamomile (*Matricaria recutita* L.) e.o. is widely used in the cosmetic industry, in aromatherapy and in traditional medicine; it is a low complexity e.o. consisting of medium-volatility components (Table 3). Chamomile e.o. is generally analysed with apolar columns (OV-1, SE-52, SE-54) in 40–50 min, e.g. analysis time with a conventional OV-1 column is about 40 min. With the corresponding short column, analysis time can be reduced to 8.5 min but α-bisabolol and α-bisabolone-oxide A coelute. Other columns were then tested to obtain a baseline separation of all the characteristic chamomile e.o.

Table 3
Retention times (t_R) and average percentages of the characteristic components of chamomile e.o. calculated over three analyses

No.	Compounds	Columns			
		SE-54 25 m		OV-1701 5 m	
		t_R (min)	Area %	t_R (min)	Area %
1	*Trans*-β-farnesene	18.80	5.9	1.37	4.8
2	Bisabolol	28.28	12.4	2.07	12.2
3	α-Bisabolol	29.94	4.4	2.15	5.4
4	α-Bisabolol oxide A	29.99	3.8	2.19	3.1
5	Bisabolol oxide A	32.84	48.4	2.33	48.0
6	Chamazulene	31.60	13.3	2.39	12.5
7	Spiroether	39.81	11.9	2.87	12.9

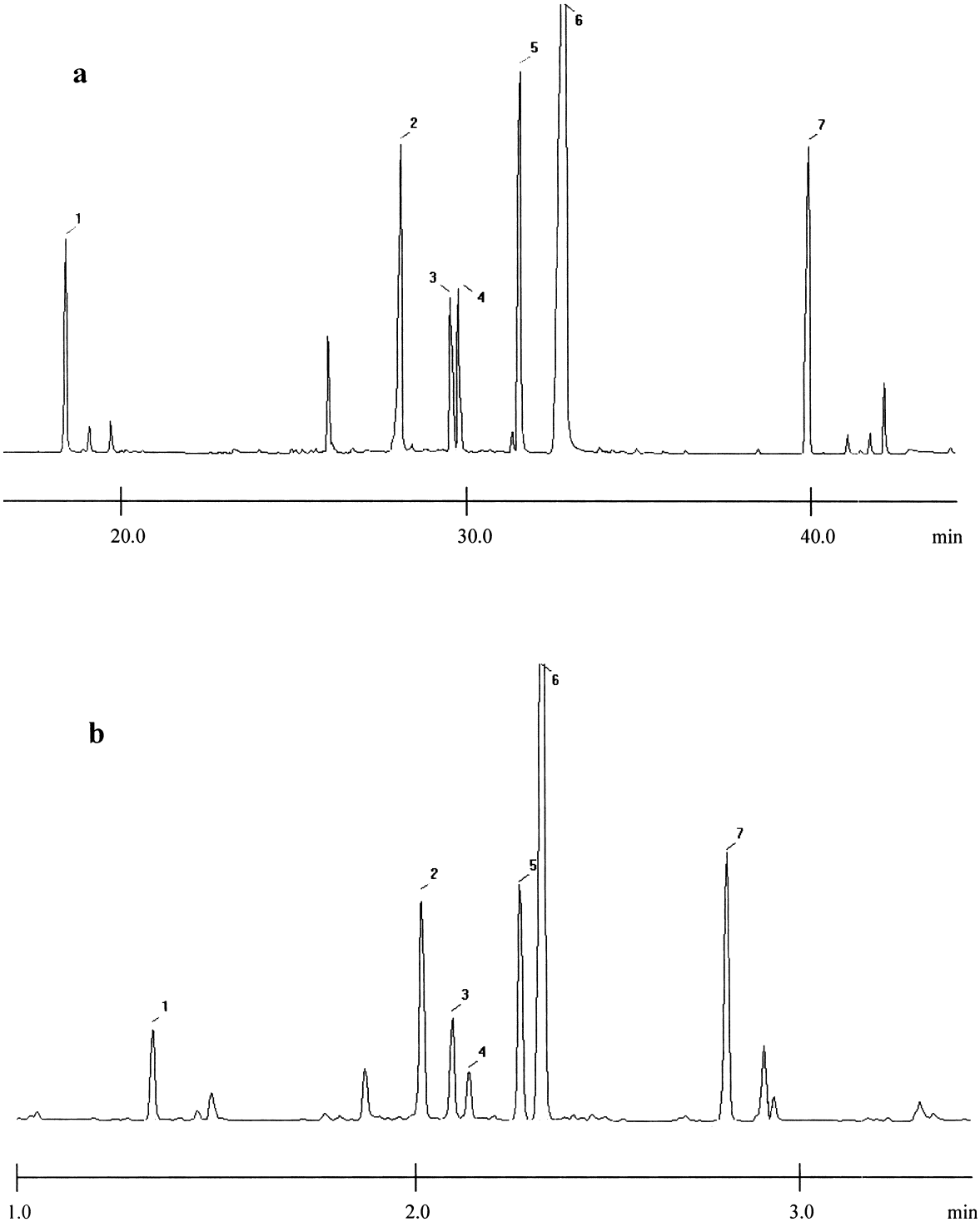

Fig. 2. cGC patterns of a chamomile e.o. obtained with the conventional SE-54 (a) and short OV-1701 (b) columns. 1: *trans*-β-farnesene, 2: bisabolol oxide B, 3: α-bisabolol, 4: β-bisabolone oxide A, 5: bisabolol oxide A, 6: chamazulene, 7: spiroether.

components, α-bisabolol and α-bisabolon-oxide included. A full separation was obtained with CW 20M, but unlike what happened with rosemary oil, this column is too polar and analysis took about 11 min. A short OV-1701 column gave the baseline separation of all analytes in less then 4 min, reducing analysis time to about one tenth of that necessary with a conventional column. Fig. 2 reports the cGC patterns of one of the investigated chamomile e.o. obtained with the conventional SE-54 (a) and short OV-1701 (b) columns. The normalized percentages of the characteristic components in the same chamomile e.o. obtained with conventional SE-54 and short OV-1701 columns were also determined. In this case too, the average percentages of each component calculated over three analyses are very similar, as is shown in Table 3. These results were confirmed by the routine analysis of 25 chamomile e.o.'s from an experimental cultivation with the above two columns.

Short columns can also contribute to reducing the analysis temperature of sample containing high-boiling components. Low analysis temperatures are particularly important for vegetable extracts, where too high a temperature can induce thermal decompositions of analytes within the column thus affecting its performance. A typical example is the analysis of friedelan-3-ol and friedelin in *Maytenus aquifolium* and *M. ilicfolium*, two brazilian plants with antiulcer activity traditionally known as ''Espinheira Santa''. These two triterpenes were analysed by high resolution GC (HRGC) and high temperature GC (HTGC) with conventional columns coated with different stationary phases (HP-5, LM-5 and OV-17) [10]. Friedelan-3-ol and friedelin elution temperatures decreased from 280°C in HRGC with HP-5 or LM-5 conventional columns, and from 290°C in HTGC with OV-17 conventional column, to 233 and 237°C, respectively, with a short SE-54 column, while the analysis time decreased from 10 or 8 min with HRGC to about 4 min, i.e. a time equivalent to that obtained with HTGC. Fig. 3 reports the cGC pattern of an extract of *Maytenus aquifolium* obtained with the short SE-54 column. The normalised percentages of friedelan-3-ol and friedelin were the same as those obtained with the above conventional columns, i.e. 63.7 and 36.3%, respectively, with a relative standard deviation (RSD%) of 0.5 calculated over ten determinations.

Short columns can also concur in the reliable analysis of thermolabile compounds. Pyrethrines are effective natural insecticides that are non-toxic to

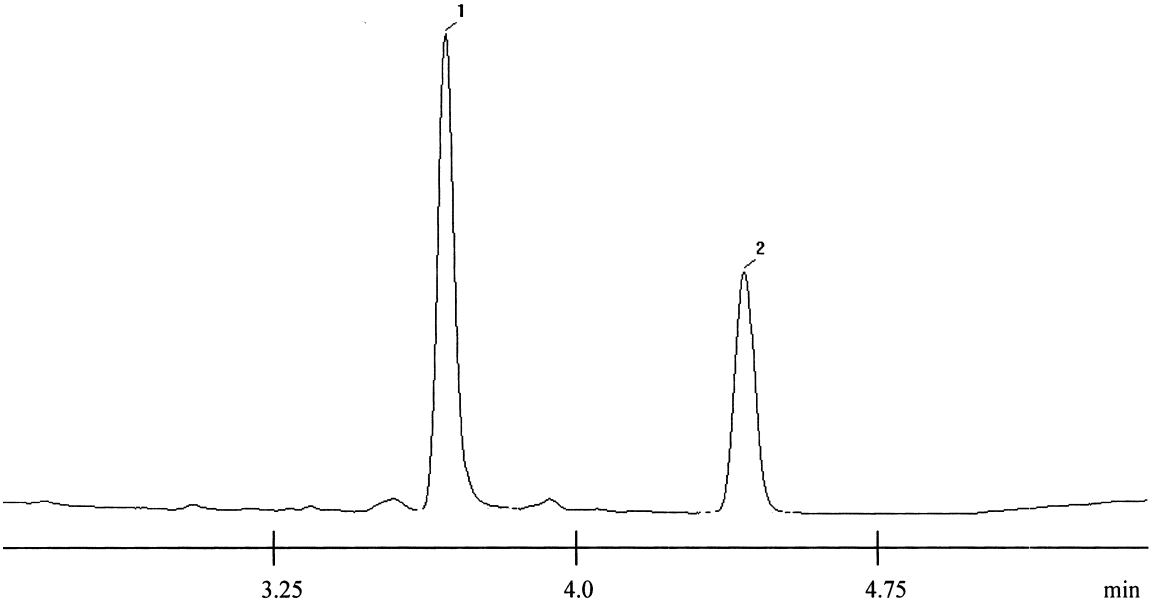

Fig. 3. cGC patterns of an extract of *Maytenus aquifolium* obtained with a short SE-54 column. 1: friedelan-3-ol, 2: friedelin.

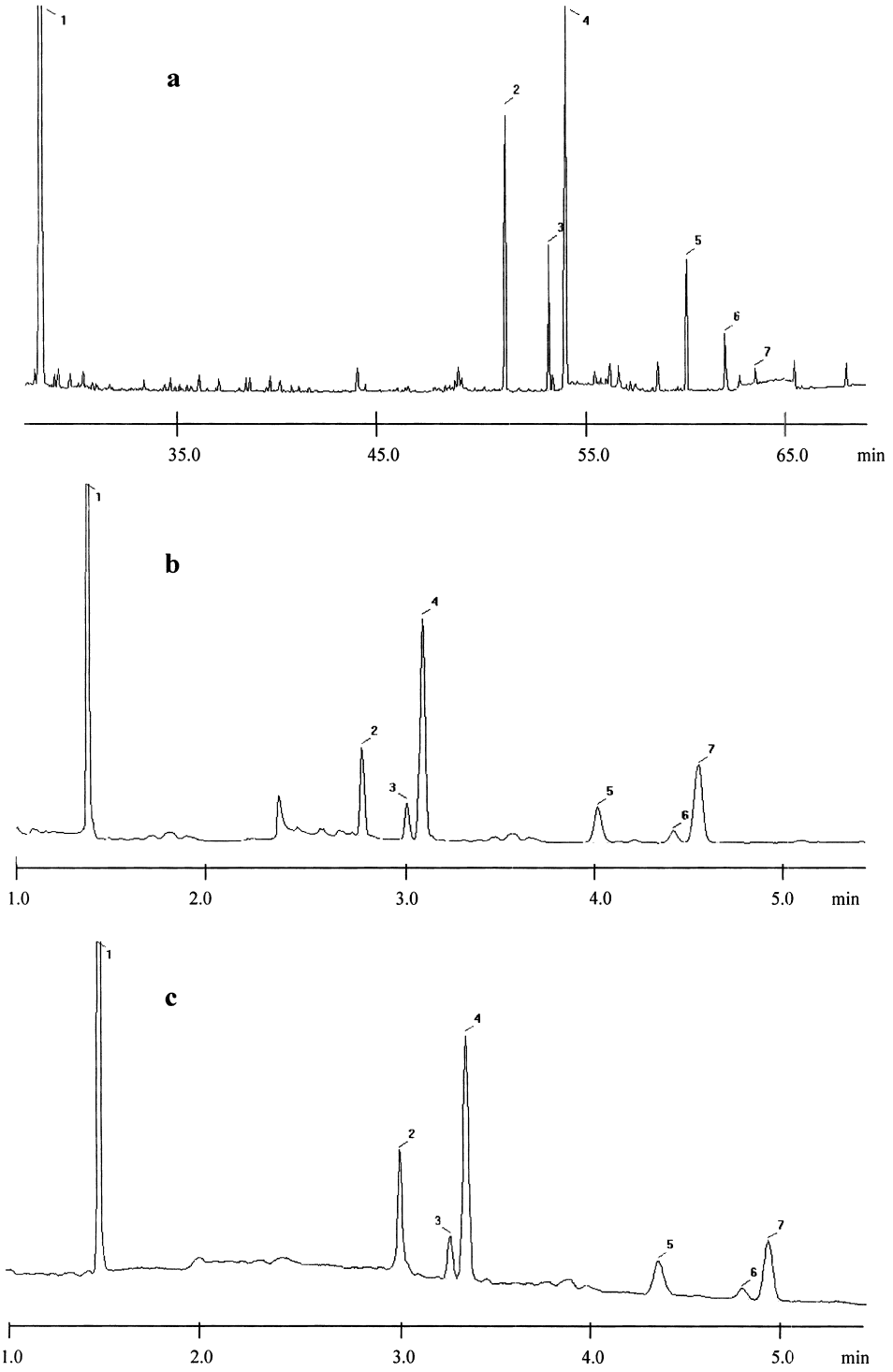

Fig. 4. cGC patterns of pyrethrins in a Pyrethrum extract analysed with: (a) split injection/conventional SE-52 column; (b) split injection/short OV-1 column; (c) cold-on-column injection/short OV-1 column. 1: ethyl dodecanoate, 2: cinerin I, 3: jasmolin I, 4: pyrethrin I, 5: cinerin II, 6: jasmolin II, 7: pyrethrin II.

Table 4
Retention times (t_R) (min), RA's of pyrethrins in a Pyrethrum extract calculated over three analyses analysed with conventional and short columns and with split and cold-on-column injection and pyrethrin mean percentages compared to that obtained by HPLC

No.	Compounds	GC columns						GC %	HPLC %
		SE-54 25 m		OV-1 5 m					
		Split		Split		On-column			
		t_R	RA	t_R	RA	t_R	RA		
1	Ethyl dodecanote	28.55	100.0	1.43	100.0	1.52	100.0	–	–
2	Cinerin I	49.44	20.7	2.82	18.0	2.97	18.6	12.8	8.8
3	Jasmolin I	51.49	9.4	3.05	7.8	3.25	9.2	6.4	5.3
4	Pyrethrin I	52.95	40.6	3.13	48.9	3.32	61.6	42.4	43.2
5	Cinerin II	60.55	9.6	4.03	11.6	4.35	16.0	11.0	9.1
6	Jasmolin II	62.32	3.4	4.41	3.6	4.72	5.8	4.0	2.9
7	Pyrethrin II	63.62	1.0	4.53	19.8	4.88	33.9	23.4	30.6

Table 5
Repeatability and reproducibility of a pesticide standard mixture: concentrations, retention times (t_R), peak areas, means and relative standard deviations (RSD%) of each pesticide investigated obtained with a short OV-1701 column together with retention time (t_R), means and relative standard deviation (RSD%) obtained with the conventional OV-1701 column

No.	Compounds	Conc. mg/ml	Columns														
			OV-1701 5 m													OV-1701 25 m		
			t_R (min)	Peak areas										Mean	RSD%	t_R (min)	Mean	RSD %
				1	2	3	4	5	6	7	8	9	10					
1	HCB	0.01	1.13	9288	10 436	10 074	10 543	11 306	9417	10 529	9723	10 195	9232	10 101	4.9	10.99	10 747	3.9
2	Lindane	0.01	1.42	9976	9994	10 601	10 006	10 910	11 134	10 276	9521	10 244	8216	10 191	4.2	12.83	10 651	3.8
3	Chlortalonil	0.1	1.65	102 127	113 109	111 451	112 249	120 772	111 924	117 090	106 359	119 101	99 159	111 676	4.9	14.23	112 805	4.0
4	Parathion methyl	0.05	1.71	74 647	84 414	82 310	80 145	89 864	79 552	84 323	74 490	81 907	72 527	80 724	4.8	14.60	81 326	4.1
5	Malathion	0.03	1.74	42 498	47 166	45 880	45 072	49 778	45 072	45 461	41 610	48 695	42 698	45 816	5.2	14.88	46 488	4.0
6	Fenitrothion	0.05	1.77	89 424	98 484	97 881	97 631	104 810	94 223	99 688	91 082	100 815	84 604	96 154	4.3	14.99	97 286	4.1
7	Parathion ethyl	0.01	1.83	24 074	26 198	26 029	26 279	27 669	25 050	26 411	24 248	26 842	22 803	25 641	4.1	15.34	26 018	4.0
8	α-Endosulfan	0.01	1.90	16 147	15 650	16 875	15 591	16 097	17 430	14 564	17 872	15 185	14 993	16 446	5.2	15.62	15 393	3.8
9	Dieldrin	0.01	2.00	15 542	17 714	17 026	17 124	18 266	16 126	17 485	15 819	17 324	14 320	16 770	4.9	16.30	17 235	4.1
10	β-Endosulfan	0.01	2.14	10 367	11 191	11 938	12 083	12 909	11 271	12 420	11 047	12 212	13 558	12 074	5.0	17.57	12 848	4.3

man and environmentally safe, and which are extracted from the dried flowers of pyrethrum (*Chrisanthemum cynerariaefolium* L.). They are mainly used for biological-crop protection and as domestic insecticides. The pyrethrins characterising a pyrethrum extracts are: pyrethrin I, pyrethrin II, cynerin I, cynerin II, jasmolin I and jasmolin II. Routinely, they are generally analysed by HPLC–UV [13] because some of them are thermolabile, in particular above 200°C pyrethrins I and II isomerize to the corresponding isoderivatives [14]. In general, thermal transformation or decomposition is related to thermal stress during injection and to residence time in the column. Pyrethrins are well separated on apolar column (SE-52) but their analysis with a conventional column takes about 1 h. With a short OV-1 column, analysis time is not only reduced to 4.5 min but also thermal decomposition is drastically limited, because the residence time in the column is shorter and the elution temperature of pyrethrins I and II is lower, i.e. 185 and 199°C instead of 205 and 237°C, respectively; moreover, their degradation can further be reduced when a cold-on-column injection system is adopted. The high constant flow-

rate applied also contributes to reducing pyrethrin elution temperatures and as a consequence their isomerisation. These results are in agreement with those reported by Class [15], who analysed pyrethrins and some pyrethroids with 10–12 m thin-film columns under constant flow or programmed pressure conditions in combination with cold-on-column injection in about 10 min showing (without reporting quantitative data) that pyrethrin I and II thermal decomposition is avoided. Fig. 4 reports those parts of the chromatograms where pyrethrins elute when analysed with conventional SE-52 (a) and short OV-1 columns (b) in combination with split injection and a short OV-1 column with cold-on-column injection (c). The reduction of thermal decomposition is also evident from the relative abundance (RA) of the labile pyrethrins calculated versus ethyl dodecanoate, taken as reference standard, and whose area was assumed equal to 100. Table 4 reports retention times and RA's of pyrethrins analysed with conventional SE-54 and short OV-1 columns with split injection and short OV-1 column with cold-on-column injection and the percentages obtained by cold-on-column injection-GC with short OV-1 column and HPLC–UV, calculated through the external standard method. From these data, it is clear that cold-on-column injection in combination with a short column makes it possible to obtain quantitative data comparable to those obtained by HPLC–UV. The pyrethrin I RA increases from about 40.6 to about 61.6 from the combination split injection/conventional column to cold-on-column injection/short column while with pyrethrin II RA increases from 1 to about 33.9 (i.e. more than 30 times). In spite of this, the percentage of pyrethrin II is still lower than that obtained by HPLC–UV, probably because a partial thermal isomerization still occurs.

Repeatability of short column results was evaluated through a standard mixture of ten pesticides widely used, among others, for medicinal plant crop protection. The list of the pesticides is reported in Table 5. These pesticides are generally baseline separated with a conventional SE-52 column in about 20 min. Short SE-52 column produces coelution of parathion-ethyl and α-endosulfan. The baseline separation of all pesticides in the mixture is obtained with a short OV-1701 column in less than 3 min. The very fast analysis time was also due to the programmed

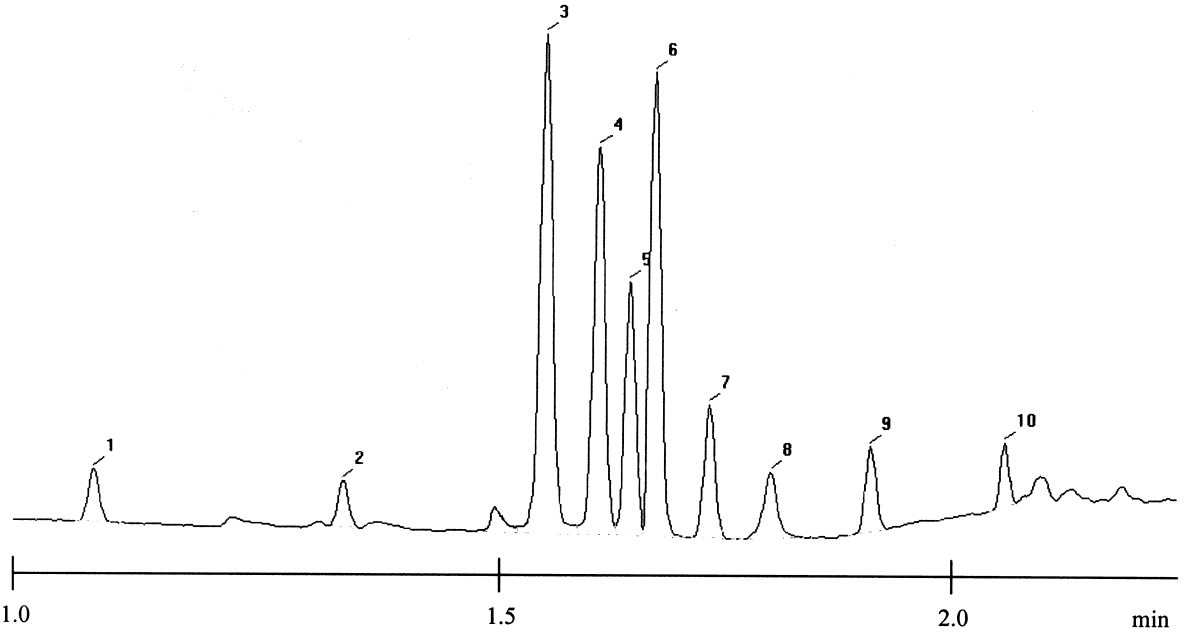

Fig. 5. cGC–FID pattern of the pesticide mixture under investigation analysed with a short OV-1701 column. 1: HCB (hexachlorobenzene), 2: lindane, 3: chlortalonil, 4: parathion methyl, 5: malathion, 6: fenitrothion, 7: parathion ethyl, 8: α-endosulfan, 9: dieldrin, 10: β-endosulfan.

flow-rate. Fig. 5 reports the GC–FID pattern of the pesticide mixture under investigation analysed with a short OV-1701 column. Repeatability was determined by analysing the standard mixture ten times each on conventional and short OV-1701 columns and by calculating mean areas and relative standard deviations for each pesticide without considering the highest and the lowest area values. Table 5 reports retention times, peak areas obtained after each analysis, mean relative standard deviations (RSD%) of each pesticide investigated obtained with the short OV-1701 column; Table 5 also reports retention times and mean areas and RSD% obtained with the conventional OV-1701 column. These results clearly show that short column results are highly repeatable since with all analytes RSDs% are around 5% and comparable to those of conventional columns. Lower RSDs% would probably have been obtained with an automatic injector. For the analysis of trace components in real-world samples, the reduced efficiency of short columns makes the use of selective GC detectors fundamental to avoid qualitative and quantitative matrix interference, in particular MS in selected ion monitoring mode (SIM-MS) becomes the ''detector'' of choice.

In conclusion, short columns with conventional I.D. diameter and coated with a suitable stationary phase are here shown to be effective in reducing analysis time of medium-to-low-complexity samples and to be complementary to narrow bore columns for fast GC analysis. The examples reported show that analysis time is shortened by factors ranging from five to ten while keeping the resolution power suitable for the complexity of the samples investigated, and that quantitative results are unaffected. The applicability of this approach for routine analysis is confirmed by a recent survey over a wide customer base that has shown that in medium-complexity samples most routine GC analyses (75–80%) quantify less than eight analytes/run and, for about half of them, there are only three or fewer analytes/run [15].

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References

[1] D.H. Desty, A. Goldup, W.T. Swanton, in: N. Brenner et al. (Eds.), Gas Chromatography, Academic Press, New York, 1962, p. 105.
[2] J.H. Knox, M. Saleem, J. Chromatogr. Sci. 7 (1969) 614.
[3] J.C. Giddings, Anal. Chem. 34 (1962) 314.
[4] C.A. Cramers, H.-G. Janssen, M.M. van Deursen, P.A. Leclercq, J.Chromatogr. A 856 (1999) 315, and references cited therein.
[5] R. Sacks, H. Smith, M. Novak, Anal. Chem. 69 (1998) 29A.
[6] F. David, D.R. Gere, F. Scanlan, P. Sandra, J. Chromatogr. A 842 (1999) 309.
[7] K. Yabumoto, W.J.A. VandenHeuvel, J. Chromatogr. 140 (1977) 197.
[8] K. Grob, in: W. Bertsch, W.G. Jennings, R.B. Kaiser (Eds.), Classical Split and Splitless Injection in Capillary GC, Huethig Verlag, Heidelberg, 1986, p. 53.
[9] European Pharmacopoeia, 3rd Edition, Council of Europe Publishing, Strasbourg, France, 1997, p. 1146.
[10] A.C. Nossack, E.C. de Vasconcelos, J.H.Y. Vilegas, F.M. Lanças, N.F. Roque, Phytochem. Anal. 11 (2000) 243, and references cited therein.
[11] L.M. Blumberg, M.S. Klee, J. Microcolumn Sep. 12 (2000) 508.
[12] Farmacopea Ufficiale della Repubblica Italiana, 10th Edition, Istituto Poligrafico e Zecca dello Stato, Roma, Italy, 1998, p. 1833.
[13] I.-H. Wang, V. Subramanian, R. Moorman, J. Burleson, J. Ko, J. Chromatogr. A 766 (1997) 277, and references cited therein.
[14] T.J. Class, J. High Resolut. Chromatogr. 14 (1991) 48.
[15] A. Sironi, Personal Communication.