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Instrumentation and applications of fast high-resolution capillary gas chromatography

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Abstract

On narrow bore GC capillary columns with 100 μm I.D., faster analyses can be performed compared to conventional GC capillary columns with 250 to 530 μm I.D., while the resolution is maintained. The use of narrow bore columns in routine analysis, however, makes high demands upon instrumentation. Recent developments in GC instrumentation, including electronic control of column flow and split flow, faster oven temperature heating and faster electronics, make fast capillary GC accessible for routine applications. Method translation software is an additional tool to translate an existing operating procedure into a narrow bore column method. The robustness of state-of-the-art fast capillary GC without impairing resolution is illustrated with the analysis of essential oils, of bacterial fatty acids, of diesel and of a polychlorinated biphenyl mixture.

Keywords: Gas chromatography, fast; Instrumentation; Oils; Diesel fuel; Fatty acids; Polychlorinated biphenyls

1. Introduction

Since the introduction of GC in 1952 [1], there has been an ongoing interest in improving separation speed. Increasing separation speed in capillary GC is in the first instance dictated by the problem at hand, the primary objective being the complete separation of the compounds in a mixture. In a large number of cases, the plate number of the capillary column is too high for a given separation problem. Consequently, the solutes are too well separated ($R \gg 2$) and the price to be paid for this ''over-resolution'' is separation speed. In these applications, resolution may be impaired. Typical ways to shorten the analysis time in this case are: decreasing the column length or increasing the carrier gas flow-rate far above the optimum. Recent alternatives include the use of multichannel columns [2] and flash GC [3].

Increasing the separation speed without impairing the resolution obtained for a given complex mixture on a certain stationary phase, can be realised by reduction of the internal diameter of the capillary column. A 10 m×0.1 mm I.D. column, for instance, offers the same resolution as a 25 m×0.25 mm I.D. column. Because the column is 2.5-times shorter, the analysis time is reduced drastically. Moreover, since the optimum carrier gas velocity is higher and the H versus u plots (Van Deemter curves) are flatter for narrow bore columns, higher average carrier gas velocities can be used without loss of resolution. The fundamental aspects of fast capillary GC using narrow bore columns have been discussed in depth in the literature [4–6] and are also presented in this thematic issue on capillary GC [7,8].

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Until now, an important obstacle for the implementation of narrow bore columns in routine capillary GC was formed by the instrumental requirements. The use of narrow bore columns requires high inlet pressures (600 kPa or higher), better split flow control, fast oven temperature heating rates and fast electronics (50 Hz or higher). Recent developments in GC instrumentation meet the requirements to apply capillary columns with internal diameters in the order of 100 μm for routine operation.

The second obstacle for the use of narrow bore columns is the need for method development and (re-)validation. Using narrow bore columns, different operational conditions (inlet pressure, split ratio, temperature program) have to be used. Since little information is yet available on the use of fast capillary GC, the transfer of standard validated operating procedures developed for conventional capillary columns into operating procedures for narrow bore columns might be difficult and definitely hamper their use in a routine environment. In this respect, the development of method translation software [9,10] is very helpful for translating a standard operating procedure for a conventional column (whatever its dimensions and stationary phase film thickness) to an operating procedure for a narrow bore column (coated with the same stationary phase). After performing the analyses on the standard column, the optimized conditions are introduced in the method translation program and all operational conditions for the new column are calculated in order to obtain the same resolution. The gain in analysis time is also predicted.

In this contribution, fast capillary GC using commercially available instrumentation is discussed for applications in which resolution is critical and cannot be impaired. The robustness of state-of-the-art fast capillary GC is illustrated with real world applications.

2. Experimental

All analyses were performed on HP 6890 gas chromatographs (Hewlett–Packard, Wilmington, DE, USA) equipped with split/splitless injection and flame ionization detection (FID) or microelectroncapture detection (μECD) detection. Automated injection was done using a HP 7673 autoinjector. Columns and operational conditions are discussed in the text. For method translation from standard columns to narrow bore columns, a dedicated software program (available free of charge from the HP Web site) was used.

3. Results and discussion

3.1. Translating optimised methods for conventional columns to narrow bore columns

For the analysis of very complex mixtures, like petrochemical samples (detailed hydrocarbon analysis of naphtha and gasolines) or flavor and fragrance samples, often very long columns (50 to 100 m) are used in order to obtain very high plate numbers (200 000 and more) and achieve ultimate resolution and peak capacity (number of peaks that can be resolved theoretically in one run). As a consequence, however, analysis times are very long as they increase proportionally with column length. Quality control (QC) of essential oil samples, for instance, is routinely done on a 50 to 60 m×0.25 mm I.D. column using a slow (2 C°/min) temperature program, which results in analysis times in the order of 2 to 3 h. The same peak capacity can be obtained on shorter narrow bore capillary columns, but, although analysis times can be reduced drastically, QC laboratories hesitate to use this approach because changing the column dimensions implies different operational conditions which results in different selectivities. Details of well-known fingerprints can be lost. The application of the method translation software allows the translation from conventional to narrow bore columns with hardly any change in resolution, selectivity and thus fingerprint. This is illustrated with the analysis of nutmeg and lemon essential oils.

The analysis of both oil samples was first performed on a ''standard'' column used for detailed essential oil profiling (60 m×0.25 mm I.D., 1 μm HP-1, phase ratio $\beta=63$, theoretical plate number= 240 000). The operational conditions optimised for routine QC were applied. Secondly, the analyses were repeated on a 20 m×0.1 mm I.D., 0.4 μm HP-1, $\beta=63$ (theoretical plate number=200 000).

The operational conditions for the narrow bore column were calculated by using the method translation software. The most important operational conditions are summarized in Table 1. From the method translation software program, a speed gain factor of 5.9 is predicted. Note that the carrier gas in the analyses are helium and hydrogen for the 250 μm I.D. and the 100 μm I.D. columns, respectively.

The chromatograms obtained for the two essential oils on the respective columns are compared in Fig. 1 [(A) nutmeg oil, standard column and (B) nutmeg oil, narrow bore column], and Fig. 2 [(A) lemon oil, standard column and (B) lemon oil, narrow bore column]. From these chromatograms, it is obvious that the resolution is very similar on both columns. The analyses on the narrow bore column are much faster. From the last eluting peak, a speed gain factor of 5.7 is measured, which is close to the predicted speed gain of 5.9. Since for the calculation, the nominal column lengths were used, it might be expected that the correlation can even be better if actual column lengths are measured and used in the method translation software.

For QC, qualitative data (programmed retention indices) and quantitative data (relative sample composition) should remain constant. The retention indices and peak areas for the most abundant peaks are compared in Tables 2 and 3 for the two essential oils, respectively. Both retention indices and quantitative data are very similar. A retention index shift of 1–2 units is normal under the large differences in temperature program rates and for not precisely measured column lengths. The reproducibility of retention times and peak areas was found to be similar for both columns. The RSDs on retention times are better than 0.1% and the RSDs on peak areas are better than 2%. The software has been applied for a wide variety of samples and performed in all cases very well. It is an indispensable tool to translate existing methods on conventional columns to narrow bore columns.

3.2. Influence of data acquisition rate

It is well known that fast capillary GC requires fast data acquisition rates. The influence of the data acquisition rate was evaluated by the analysis of a mixture of bacterial fatty acid methyl esters. High speed analysis of fatty acid methyl esters is best performed on selective columns (biscyanopropyl silicone or polyethylene glycol), because the greater the stationary phase selectivity, the faster the analysis can be performed [5,11,12]. For the characterisation of bacteria, however, the profile of the cellular fatty acids obtained on an apolar column is preferred because reference libraries are available [13,14]. The analyses were performed on a 10 m×0.10 mm I.D., 0.1 μm HP-5. One μl of a test sample (BAME mixture 4-7080; Supelco, Bellefonte, PA, USA) was injected at 300°C in the split mode (1/50). Carrier gas was hydrogen at 276 kPa (70 cm/s linear velocity at 150°C) The oven was programmed from 150°C to 250°C at 20 C°/min.

The analysis is shown in Fig. 3. All compounds are separated in less than 3.6 min. In comparison to published data on conventional apolar capillary

Table 1
Experimental conditions for essential oil analysis on a conventional and on a narrow bore column

Column	60 m×0.25 mm I.D., 1 μm HP-1	20 m×0.1 mm I.D., 0.4 μm HP-1
Injection	Split, 1 μl, 1/50 split ratio, 250°C	Split, 1 μl, *1/500* split ratio, 250°C
Carrier type	Helium	Hydrogen
Carrier pressure	209 kPa, constant pressure	*411 kPa*, constant pressure
Carrier flow	1.74 ml/min at 50°C	*0.87 ml/min* at 50°C
Velocity	29.5 cm/s (average at 50°C)	*58 cm/s* (average at 50°C)
Hold-up time	3.39 min	*0.57 min*
Oven program	50°C–2 C°/min–275°C–40 min	*50°C–11.88 C°/min–275°C–7 min*
Detection	FID	FID
Signal data rate	10 Hz	50 Hz
Analysis time	152.5 min	*25.94 min*

The conditions in italic for the narrow bore column have been calculated by the method translation software. The standard operating conditions (SOP) on the conventional column apply broader conditions than needed for the two essential oils analysed in this contribution.

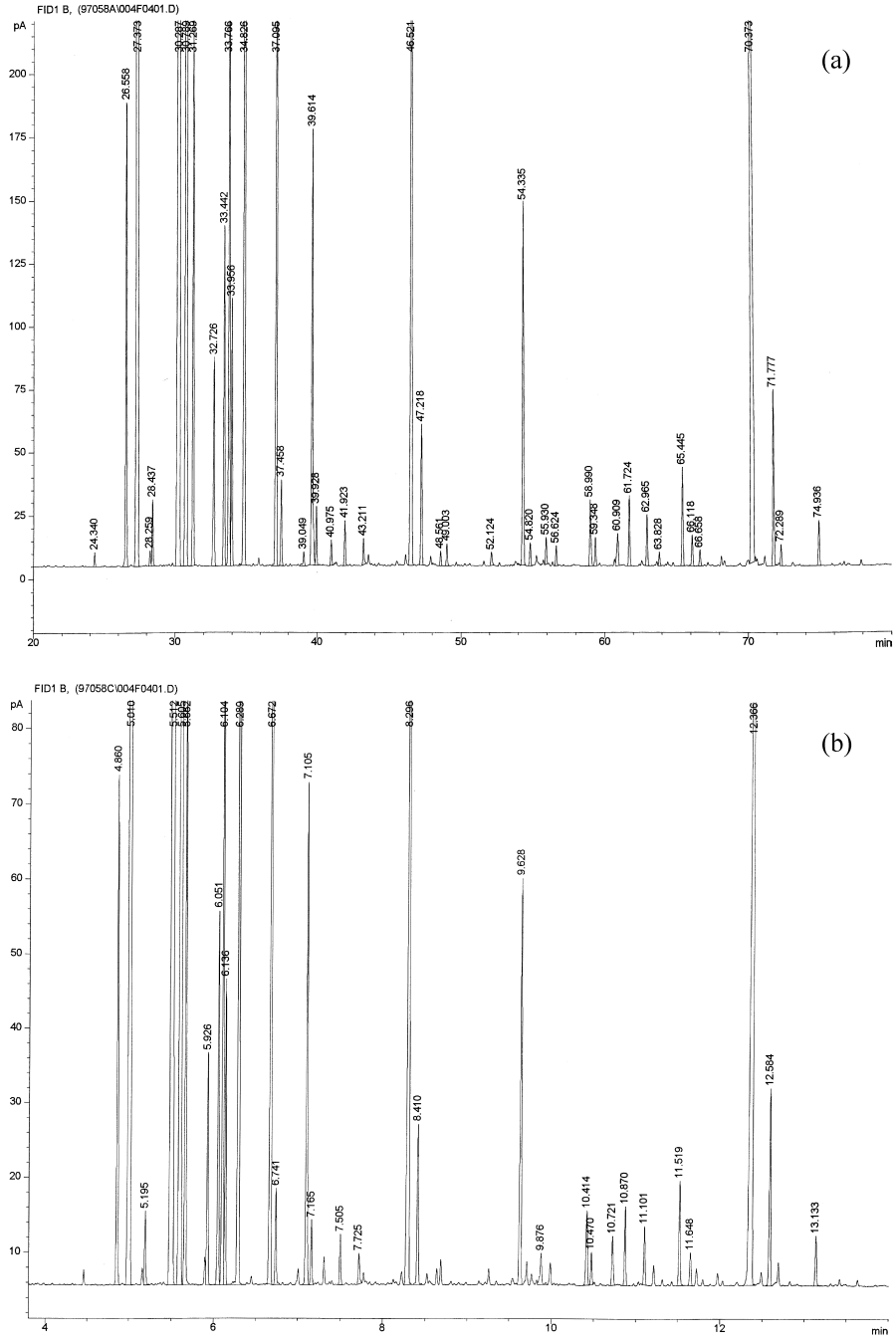

Fig. 1. Analysis of nutmeg oil on (A) a 60 m×0.25 mm I.D., 1 μm HP-1 column, (B) a 20 m×0.1 mm I.D., 0.4 μm HP-1 column.

columns [11,12], this is a speed gain by a factor of 10. The retention time and peak area repeatabilities were calculated for ten runs, using respectively 5 Hz and 50 Hz sampling rates for FID. The results are given in Table 4. From these data, the necessity of using fast electronics for data acquisition in fast gas

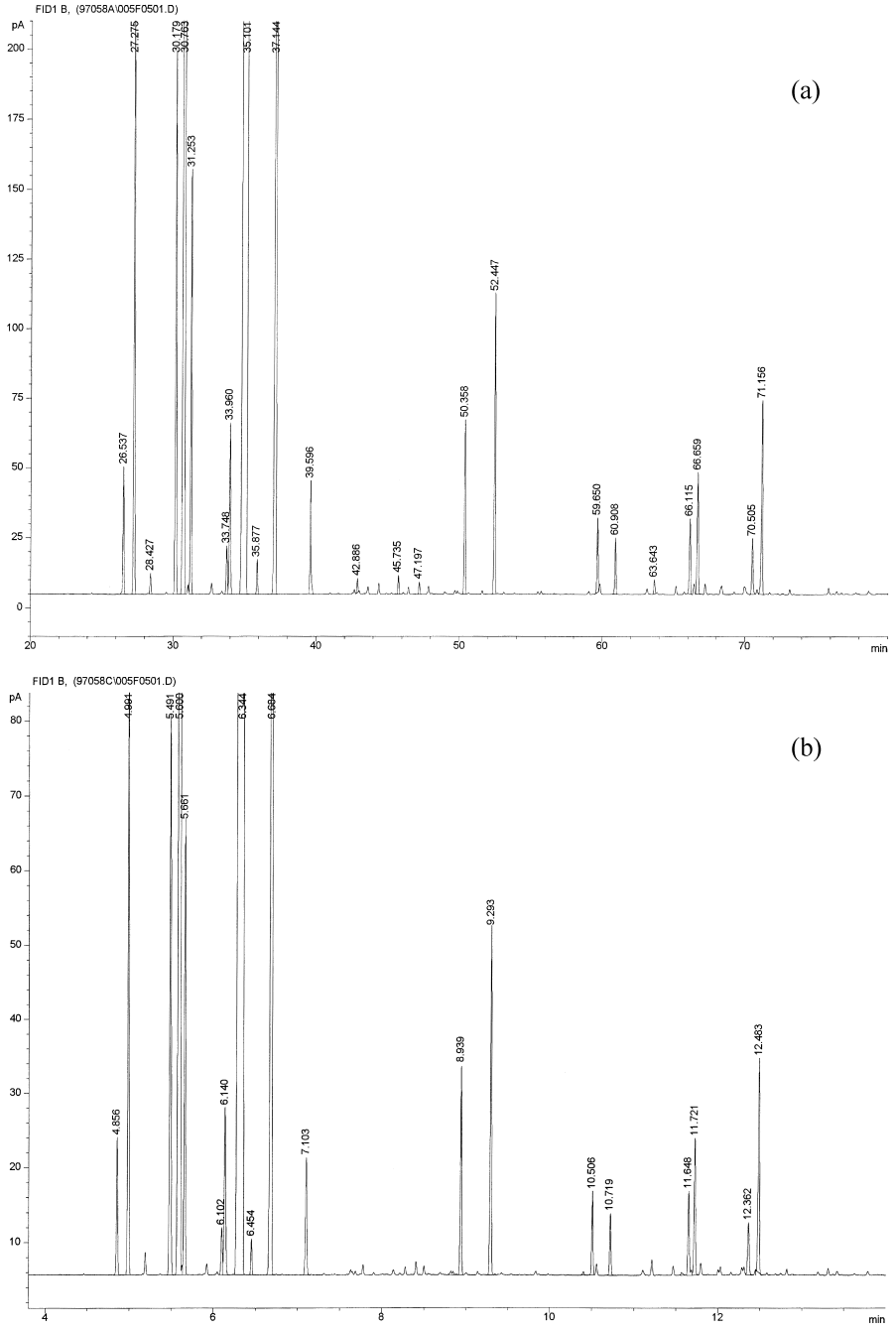

Fig. 2. Analysis of Californian lemon oil on (A) a 60 m×0.25 mm I.D., 1 μm HP-1 column, (B) a 20 m×0.1 mm I.D., 0.4 μm HP-1 column.

Table 2
Comparison of retention times, retention indices (*I*) and relative peak areas obtained on both columns for the analysis of nutmeg oil

Peak	60 m×0.25 mm I.D., 1 μm HP-1			20 m×0.1 mm I.D., 0.4 μm HP-1		
	t_R (min)	*I*	Area %	t_R (min)	*I*	Area %
1	27.373	936	19.8	5.011	938	19.8
2	30.287	971	22.5	5.513	973	22.6
3	30.789	977	13.8	5.606	979	13.9
4	31.269	983	2.6	5.663	983	2.6
5	33.766	1013	2.8	6.104	1014	2.8
6	34.826	1026	6.5	6.289	1027	6.6
7	37.095	1053	4.4	6.673	1054	4.5
8	39.614	1083	2.1	7.105	1084	2.1
9	46.521	1168	4.9	8.296	1171	5.0
10	70.373	1495	7.8	12.367	1498	8.2

chromatography is obvious. Using a 5 Hz rate, the standard deviation on retention times is better than 0.002 min, while this is better than 0.001 min if a 50 Hz rate is used. Comparing the standard deviation on peak areas, the difference is even more pronounced. With a 50 Hz rate, the RSD is better than 2%, except for the hydroxy fatty acids (RSD=2–4%). Using a 5 Hz sampling rate, the RSDs are higher than 4% for most compounds and even higher than 10% for some compounds.

3.3. Influence of oven heating rate

Fast capillary GC requires fast oven temperature ramps. Standard GC equipment generally allows program rates up to 40 C°/min. Although higher rates can be programmed, it is found that actual oven temperature deviates from programmed temperatures. Since the heating (and cooling) of the oven also depends on the oven dimensions, reducing the oven size allows faster ramping.

The influence of the oven size is illustrated with the analysis of diesel. Prerequisite in the development of fast capillary GC for this application, was the baseline separation of the biomarkers pristane and phytane from the preceding normal hydrocarbons. First the analysis was performed using the standard HP 6890 oven. Secondly, an oven insert (pillow) was placed in the oven, reducing its size by 50%. The insert is placed on the front side (door side), so the back inlet and detector are used. A diesel sample was diluted ten times in cyclohexane

Table 3
Comparison of retention times, retention indices (*I*) and relative peak areas obtained on both columns for the analysis of lemon oil

Peak	60 m×0.25 mm I.D., 1 μm HP-1			20 m×0.1 mm I.D., 0.4 μm HP-1		
	t_R (min)	*I*	Area %	t_R (min)	*I*	Area %
1	27.275	935	2.16	4.992	936	2.15
2	30.179	970	2.15	5.491	971	2.14
3	30.763	977	14.23	5.603	979	14.19
4	31.253	983	1.42	5.661	983	1.43
5	33.960	1015	0.70	6.139	1017	0.70
6	35.101	1029	65.44	6.349	1031	65.47
7	37.144	1053	8.65	6.686	1055	8.64
8	50.358	1117	0.64	8.940	1119	0.71
9	52.447	1144	1.14	9.294	1146	1.21
10	71.156	1507	0.76	12.483	1509	0.79

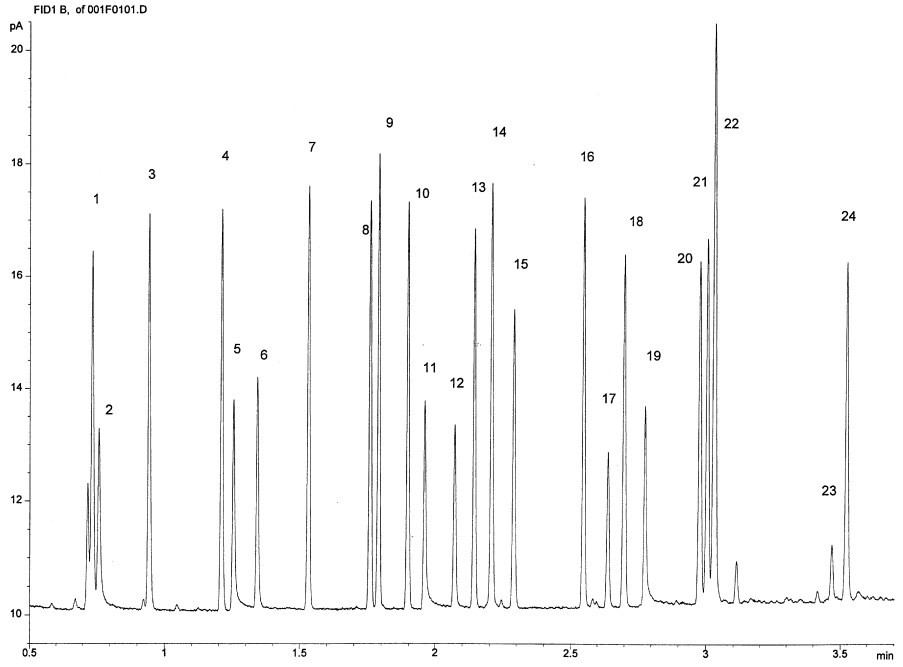

Fig. 3. Analysis of bacterial fatty acid methyl esters on a 10 m×0.1 mm I.D., 0.1 μm HP-5 column (for peak identification, see Table 4).

and 1 μl was injected in the split mode (1/100 split ratio). The column was a 10 m×0.1 mm I.D., 0.1 μm HP-1. The oven was programmed from 100°C to 325°C at 75 C°/min and hold for 1 min (4 min program). FID with a signal data acquisition rate of 100 Hz was used. The actual oven temperature was monitored during the analyses.

The chromatograms obtained with and without oven insert are compared in Fig. 4. The monitored actual oven temperatures with and without oven insert are shown in Fig. 5. Reducing the oven dimensions gives a reduction in analysis time of 4 s for C_{28}. With the standard oven size, the actual temperature starts to deviate from 250°C (after 2 min). With the oven insert, no deviation was observed. Although the retention time reproducibility was found to be equal in both sets of data (SDs= 0.001 min), application of method translation was found to be more accurate with the reduced oven size.

An important aspect for routine analysis (i.e. process control) is the oven recycle time. A sequence of ten diesel analyses using the above mentioned conditions required a total analysis time (time at end of tenth run−start of sequence) of 76 min 24 s. With the oven insert, the total time was 72 min 5 s, or a 4.5 min gain.

3.4. Sample introduction in high speed capillary GC

The sample capacity and volume loadability of narrow bore columns is much smaller compared to conventional columns. Moreover, automated injection is mandatory. For split injection, the split ratio is increased to avoid overloading and peak fronting (calculated by the method translation software). Sensitivity is hereby not lost, since the sensitivity of mass sensing detectors such as FID, depends on the amount (ng) of solute per unit of time reaching the detector. The peak widths are smaller on the narrow bore column and the overall signal-to-noise is nearly constant [8].

Narrow bore columns can also be used in combination with splitless or programmed temperature vaporizing (PTV) (in splitless or solvent vent mode)

Table 4
Reproducibility of retention times and peak areas for the analysis of bacterial fatty acid methyl esters as a function of data acquisition rate

Peak	BAMEs	5 Hz				50 Hz			
		t_R		Area		t_R		Area	
		Mean (min)	RSD (%)	Mean (counts)	RSD (%)	Mean (min)	RSD (%)	Mean (counts)	RSD (%)
1	Me undecanoate	0.743	0.04%	2.245	8.28%	0.738	0.00%	4.065	0.48%
2	Me 2-OH-decanoate	0.768	0.06%	1.085	6.65%	0.763	0.00%	2.317	2.23%
3	Me dodecanoate	0.951	0.07%	2.267	3.27%	0.947	0.05%	4.212	1.51%
4	Me tridecanoate	1.219	0.04%	2.157	5.11%	1.214	0.04%	4.201	0.37%
5	Me 2-OH-dodecanoate	1.263	0.03%	1.163	4.92%	1.258	0.00%	2.401	1.91%
6	Me 3-OH-dodecanoate	1.350	0.05%	1.225	7.67%	1.346	0.04%	2.415	0.84%
7	Me tetradecanoate	1.540	0.05%	2.233	5.35%	1.535	0.03%	4.405	0.34%
8	Me 13-Me-tetradecanoate	1.766	0.04%	2.114	7.23%	1.762	0.02%	4.227	0.30%
9	Me 12-Me-tetradecanoate	1.797	0.04%	2.503	6.88%	1.793	0.02%	4.475	0.47%
10	Me pentadecanoate	1.904	0.04%	2.528	20.73%	1.900	0.03%	4.289	0.44%
11	Me 2-OH-tetradecanoate	1.968	0.04%	1.763	24.01%	1.963	0.02%	2.413	2.83%
12	Me 3-OH-tetradecanoate	2.079	0.04%	1.074	13.90%	2.074	0.02%	2.004	1.56%
13	Me 14-Me-pentadecanoate	2.150	0.03%	1.949	7.09%	2.146	0.02%	3.973	1.57%
14	Me *cis*-9-hexadecenoate	2.214	0.03%	2.792	5.74%	2.210	0.02%	4.769	0.64%
15	Me hexadecanoate	2.297	0.04%	1.747	4.98%	2.292	0.03%	3.402	0.38%
16	Me 15-Me-hexadecanoate	2.554	0.03%	2.438	6.27%	2.550	0.02%	4.638	0.55%
17	Me c-9,10-methylenehexadecanoate	2.646	0.05%	1.219	4.33%	2.641	0.02%	1.803	1.24%
18	Me heptadecanoate	2.705	0.03%	2.225	4.06%	2.701	0.02%	4.282	0.46%
19	Me 2-OH-hexadecanoate	2.784	0.03%	2.139	5.93%	2.779	0.02%	2.357	2.41%
20	Me c-9,12-octadecadienoate	2.983	0.03%	2.233	6.97%	2.979	0.02%	4.158	0.69%
21	Me c-9-octadecenoate	3.011	0.03%	3.293	4.02%	3.005	0.01%	4.439	1.09%
22	Me c-11-octadecenoate	3.035	0.04%	4.647	3.03%	3.031	0.02%	7.700	0.44%
23	Me c-9,10-methyleneoctadecanoate	3.472	0.02%	1.315	20.51%	3.468	0.01%	0.066	4.01%
24	Me nonadecanoate	3.528	0.02%	2.805	8.15%	3.523	0.02%	4.155	0.75%

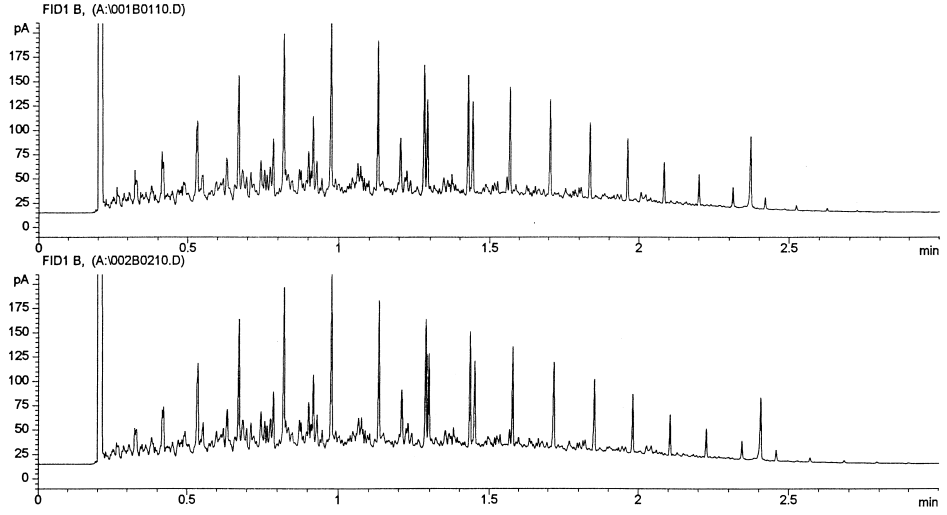

Fig. 4. Analysis of diesel by fast capillary GC using a reduced size GC oven (top) and a standard GC oven (bottom).

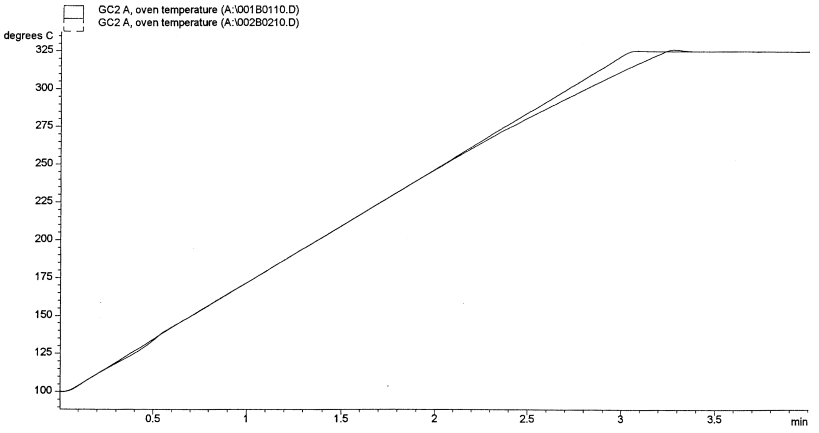

Fig. 5. Actual oven temperature during the analysis of diesel by fast CGC using a reduced size GC oven (top line) and a standard GC oven (broken line).

injection. This is illustrated by the analysis of polychlorinated biphenyls (PCBs) using capillary GC–ECD. First the analysis of a PCB mixture dissolved in hexane was performed on a 30 m×0.25 mm I.D., 0.25 μm HP-5 column. The analysis was then repeated on a 10 m×0.1 mm I.D., 0.1 μm HP-5 column. The operational conditions for the narrow bore column were calculated using the method translation software. The most important operational conditions are summarized in Table 5. From the method translation software program, a speed gain factor of 2.96 is predicted.

The chromatograms obtained on both columns are given in Fig. 6. From these chromatograms, it is clear that for 1 μl splitless injection, resolution is maintained. The last PCB congener is eluting at 10.285 min on the narrow bore column, versus 30.065 min on the standard column. This is a speed gain of 2.92, corresponding very well to the predicted factor. Splitless injection on the narrow bore column did not deteriorate peak shape.

4. Conclusion

New developments in GC instrumentation, including electronic pneumatic control of the column head pressure and of the split flow, fast and reproducible oven heating and faster electronics, offer new possibilities for the implementation of narrow bore columns (100 μm I.D.) in routine QC. Using the method translation software, an existing operating procedure for a standard capillary column can be translated into an operating procedure for a narrow bore column, resulting in a faster analysis with the same resolution. Both qualitative and quantitative data remain unaffected.

Table 5
Experimental conditions for analysis of a PCB mixture

Column	30 m×0.25 mm I.D., 0.25 μm HP-5	10 m×0.1 mm I.D., 0.1 μm HP-5
Injection	Splitless, 1 μl, 250°C, 0.75 min splitless time	Splitless, 1 μl, 250°C, 0.5 min splitless time
Carrier type	Hydrogen	Hydrogen
Carrier pressure	51 kPa at 50°C	*177 kPa* at 50°C
Carrier flow	1.2 ml/min constant flow mode	*0.5 ml/min* constant flow mode
Velocity	35 cm/s (average at 50°C)	*55 cm/s* (average at 50°C)
Oven program	50°C–1 min–40 C°/min–150°C–4 C°/min–270°C–5 min	*50°C–1 min–40 C°/min–150°C–14.2 C°/min–270°C–1.06 min*
Detection	μECD, 320°C	μECD, 320°C
Analysis time	38.5 min	*13.01 min*

The conditions in italic for the narrow bore column have been calculated by the method translation software.

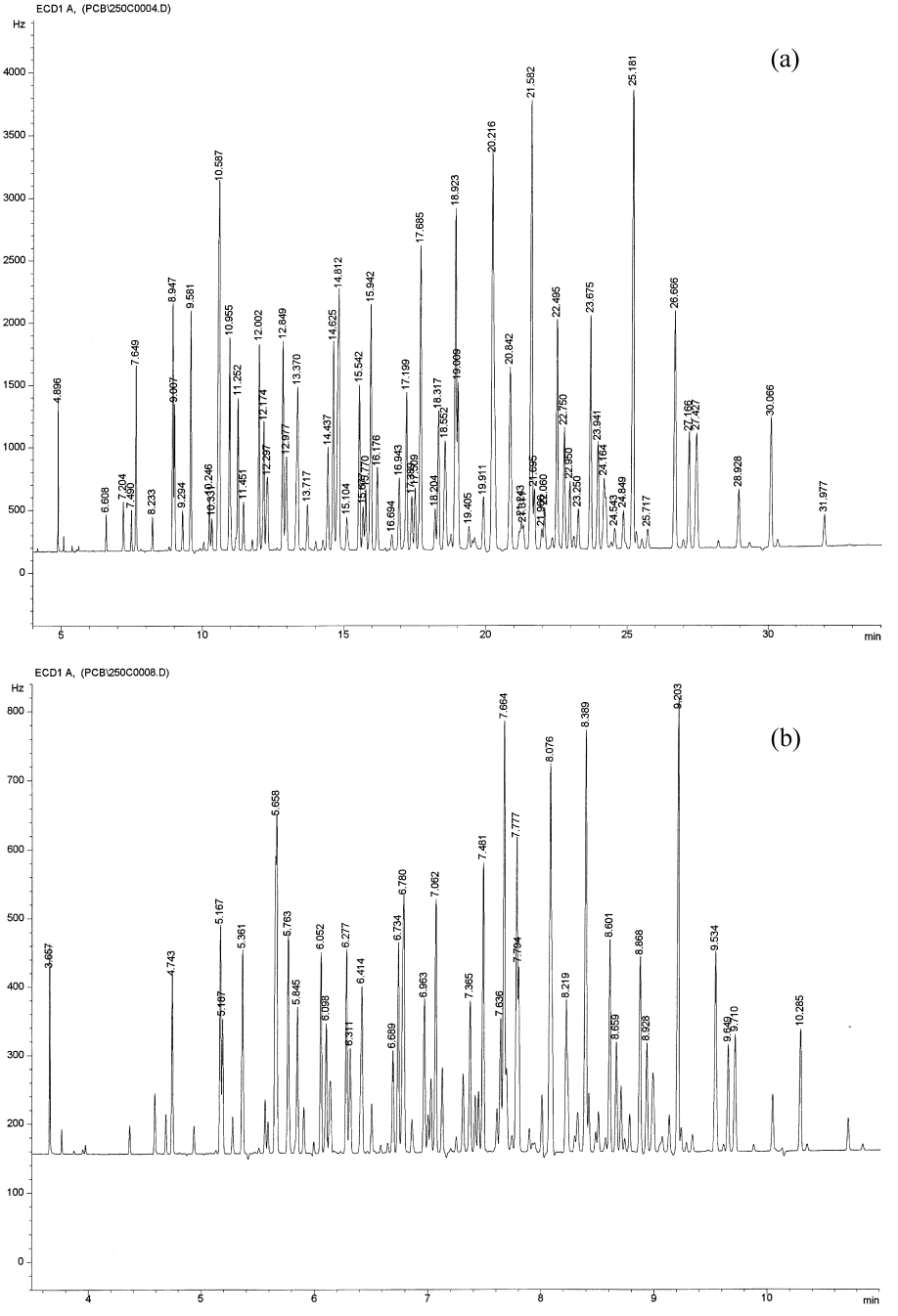

Fig. 6. Analysis of PCB mixture on a 30 m×0.25 mm I.D., 0.25 μm HP-5 column (top) and on a 10 m×0.1 mm I.D., 0.1 μm HP-5 narrow bore column (bottom).

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