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Development of fast enantioselective gas-chromatographic analysis using gas-chromatographic method-translation software in routine essential oil analysis (lavender essential oil)

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ABSTRACT

The study aimed to find the best trade-off between separation of the most critical peak pair and analysis time, in enantioselective GC–FID and GC–MS analysis of lavender essential oil, using the GC method-translation approach. Analysis conditions were first optimized for conventional 25 m × 0.25 mm inner diameter (d_c) column coated with $6^{I–VII}$-*O*-*tert*-butyldimethylsilyl-$2^{I–VII}$-$3^{I–VII}$-*O*-ethyl-β-cyclodextrin (CD) as chiral stationary phase (CSP) diluted at 30% in PS086 (polymethylphenylpolysiloxane, 15% phenyl), starting from routine analysis. The optimal multi-rate temperature program for a pre-set column pressure was determined and then used to find the pressures producing the efficiency-optimized flow (EOF) and speed-optimized flow (SOF). This method was transferred to a shorter narrow-bore (NB) column (11 m × 0.10 mm) using method-translation software, keeping peak elution order and separation. Optimization of the enantioselective GC method with the translation approach markedly reduced the analysis time of the lavender essential oil, from about 87 min with the routine method to 40 min with an optimal multi-rate temperature program and initial flow with a conventional inner diameter column, and to 15 min with FID as detector or 13.5 min with MS with a corresponding narrow-bore column, while keeping enantiomer separation and efficiency.

1. Introduction

One of the approaches used to meet the increasing demand for routine chiral recognition of real-world samples is to speed up enantioselective GC (Es-GC), thus increasing sample throughput, laboratory productivity and, as a consequence, reducing analysis costs. Cyclodextrin derivatives (CDs) are the most widely used chiral selectors in Es-GC in the flavour and fragrance field [1,2]. Chiral recognition with CDs is due to the small difference in the energy of the host/guest interactions between each enantiomer and the chiral selector, and is entirely governed by thermodynamics [3,4]; in consequence it is closely controlled by temperature. The decisive contribution of temperature to chiral discrimination limits the heating rates that can be applied, and makes column length, inner diameter and/or carrier gas and flow rate the most important parameters on which to act to speed up an enantiomer GC separation. In a previous article, Bicchi et al. [5] successfully applied short conventional and narrow-bore CD columns to speeding up Es-GC analysis of real-world samples in the essential oil field, by applying temperature rates up to 10 °C/min and using mass spectrometry (MS) as a further dimension of discrimination, i.e. to overcome peak co-elution due to column shortening and increased heating rates. A resolution limit of 1.5 was assumed to enable correct enantiomeric excess (ee) and/or enantiomeric ratio (er) determination. The lower enantiomer elution temperatures due to short columns increased CD enantioselectivity and (at least partially) compensated for the loss of efficiency due to column shortening. The study aimed to achieve the best trade-off between analysis speed and loss of resolution of chiral compounds, without considering the effect on the total separation compensated by the MS action. It started from routine analysis conditions usually applied to conventional inner diameter (0.25 mm) and length (25 m) columns coated with $6^{I–VII}$-*O*-*tert*-butyldimethylsilyl(TBDMS)-$2^{I–VII}$,$3^{I–VII}$-*O*-ethyl-β-cyclodextrin diluted at 30% in PS-086 (polymethylphenylsiloxane, 15% phenyl) and with a helium flow rate of 1.0 mL/min.

In general, a laboratory routinely analysing numerous different samples in a single field (e.g. essential oils from different plants) tends to adopt the same standardized GC conditions for all of them, rather than optimizing the method for each matrix; this approach also enables automatic peak identification from chromatographic data (relative retention times, linear retention indices, etc.). More-

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Table 1
Retention time (t_R) and resolution (R_s) of the enantiomers of the lavender e.o. chiral markers under two initial flow rate and a several single-ramp heating rates. Conditions: conventional column, FID.

Initial flow rate (mL/min)		1		2		2		2		2	
Heating rate (°C/min)		2.0		2.0		2.6		3.3		5.0	
Compound		t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s
1a	(*S*)-α-Pinene	13.14	1.0	9.84	1.0	8.70	1.0	7.85	0.9	6.45	0.6
1b	(*R*)-α-Pinene	13.02		9.74		8.62		7.78		6.41[1]	
2a	(*S*)-Camphene	12.90	7.5	9.52	6.9	8.49	6.2	7.70	6.8	6.41[1]	5.0
2b	(*R*)-Camphene	13.65		10.21		9.04		8.15		6.71	
3a	(*S*)-β-Pinene	14.93	4.5	11.33	4.9	9.96	4.4	8.92	4.0	7.27	3.3
3b	(*R*)-β-Pinene	14.40		10.82		9.56		8.60		7.06	
4a	(*S*)-β-Phellandrene	20.85	5.1	16.88	5.9	14.38	5.5	12.49	4.9	9.64[2]	2.7
4b	(*R*)-β-Phellandrene	20.22		16.20		13.87		12.11		9.48	
5a	(*S*)-Limonene	20.65	6.5	16.65	6.8	14.21	6.7	12.37	6.5	9.64[2]	6.0
5b	(*R*)-Limonene	21.55		17.48		14.91		12.92		9.98	
6	1-Octen-3-ol	24.43	NR	20.67	NR	17.22	NR	14.67	NR	11.04	NR
7b	(*R*)-Camphor	25.86	1E	21.40	1E	18.02	1E	15.51	1E	11.88	1E
8a	(*S*)-Linalool	28.23	6.3	24.26[1]	7.0	20.05	6.1	16.96	5.5	12.63	4.1
8b	(*R*)-Linalool	27.28		23.27		19.28		16.37		12.23	
9a	(*S*)-Borneol	28.86	2.9	24.26[1]	4.5	20.30	3.5	17.33	2.7	13.10	2.3
9b	(*R*)-Borneol	29.30		24.80		20.66		17.59		13.25	
10a	(*S*)-Linalyl acetate	31.54	2.0	26.92	3.0	22.34	2.6	18.99	2.0	14.07[3]	NR
10b	(*R*)-Linalyl acetate	31.20		26.53		22.06		18.74		14.07[3]	
11a	(*S*)-Terpinen-4-ol	31.84	2.0	27.50	2.2	22.68	2.2	19.14	1.9	14.21	1.6
11b	(*R*)-Terpinen-4-ol	32.12		27.80		22.91		19.31		14.32	
12b	(*R*)-Lavandulyl acetate	33.02[1]	1E	28.17	1E	23.37	1E	19.83	1E	14.84	1E
13b	(*R*)-Lavandulol	33.02[1]	1E	29.02	1E	23.74	1E	19.87	1E	14.59	1E
14a	(*S*)-α-Terpineol	34.53	5.0	30.24	6.1	24.78	5.5	20.77	4.9	15.27	3.9
14b	(*R*)-α-Terpineol	35.19		30.96		25.30		21.17		15.52	

[1,2,3] coeluting peaks; 1E = only one enantiomer detected; NR = not resolved.

over, satisfactory separations are usually obtained because the chromatographic system provides an efficiency much higher than is required, although this excess is generally paid for in terms of analysis times that are longer than they need to be. Optimization of analysis conditions of a given sample can successfully and drastically speed up a routine GC analysis. The main question is how to optimize a method without losing separation and keeping the same order of analyte elution. This topic was investigated in depth by Blumberg and co-workers in a series of studies highlighting the most important theoretical concepts to optimize capillary GC methods and achieve the best speed/separation trade-off [6–10]. The result was the well-known GC method-translation [11]—a method optimization approach that preserves the peak elution order. In GC method-translation, the parameters influencing the analysis are divided into two main groups: translatable and non-translatable. Stationary phase type and phase ratio are non-translatable column parameters. All other column and method parameters including column dimension (d_c and length), outlet pressure (1 atm for FID, vacuum for MS, etc.), carrier gas and flow rate (F), are translatable [10]. This approach adopts the hold-up time as time unit to express all time-related parameters, including the duration of temperature plateau(s) and heating rate(s), which, in turn, leads to a normalized temperature programme for the analysis considered. As a result, two methods are translatable when they have identical non-translatable parameters and normalized temperature programmes. For a given temperature-programmed analysis, thanks to the method-translation principles, it is possible to optimize either the flow rate producing the highest efficiency (i.e. the plate number) of a given column (efficiency-optimized flow, EOF), or a combination of flow rate, column dimensions and carrier gas type that corresponds to the shorter analysis time for a given required plate number (speed-optimized flow, SOF [8]). Method-translation software is available free of charge from the Internet [12].

This study sought the best trade-off between separation of the most critical peak pairs and total efficiency of the chromatographic system, while shortening analysis time, in the enantioselective analysis of the lavender essential oil in a conventional d_c column, and then the optimized method was transferred to a shorter narrow-bore column. Suitable GC method-translation was used in the method modifications to preserve the peak elution order during flow rate optimization in the conventional bore column, and to keep that elution order in the shorter narrow-bore column with FID and MS as detectors. Lavender oil was again used as model sample because it is characterized by a large number of chiral markers [13] and their enantiomeric composition in a genuine oil is reliably described in the literature [14].

2. Experimental

2.1. Samples

Pure standards of nonane, decane, undecane and racemic α-pinene, limonene and linalool, were from the collection of standards in the authors' laboratory. All standard compounds were solubilised in cyclohexane at a concentration of 100 mg/L each. Solvents were all HPLC grade from Riedel-de Haen (Seelze, Germany). Lavender (*Lavandula angustifolia* P. Mill.) essential oil (e.o.), obtained by hydrodistillation following the method described in the European Pharmacopoeia (6th edition) [13], was diluted 1:200 in cyclohexane before analysis.

2.2. Instrumental set-up

A Shimadzu GC 2010 system (Shimadzu, Milan, Italy) provided with Shimadzu GC Solution 2.53SU1 software and an Agilent 6890 GC system (Agilent, Little Falls, DE, USA) with Agilent—LC/MSD ChemStation (version A.08.03–847) software were used for the GC–FID analyses. Agilent 6890-5975 GC–MS with an Agilent—MSD ChemStation version D.02.00.275 software was used for the GC–MS analyses.

Columns: GC analyses were carried out on two columns coated with $6^{I–VII}$-*O*-TBDMS-$2^{I–VII}$-$3^{I–VII}$-*O*-ethyl-β-CD [15] as chiral stationary phase (CSP) diluted at 30% in PS086: a 25 m conventional

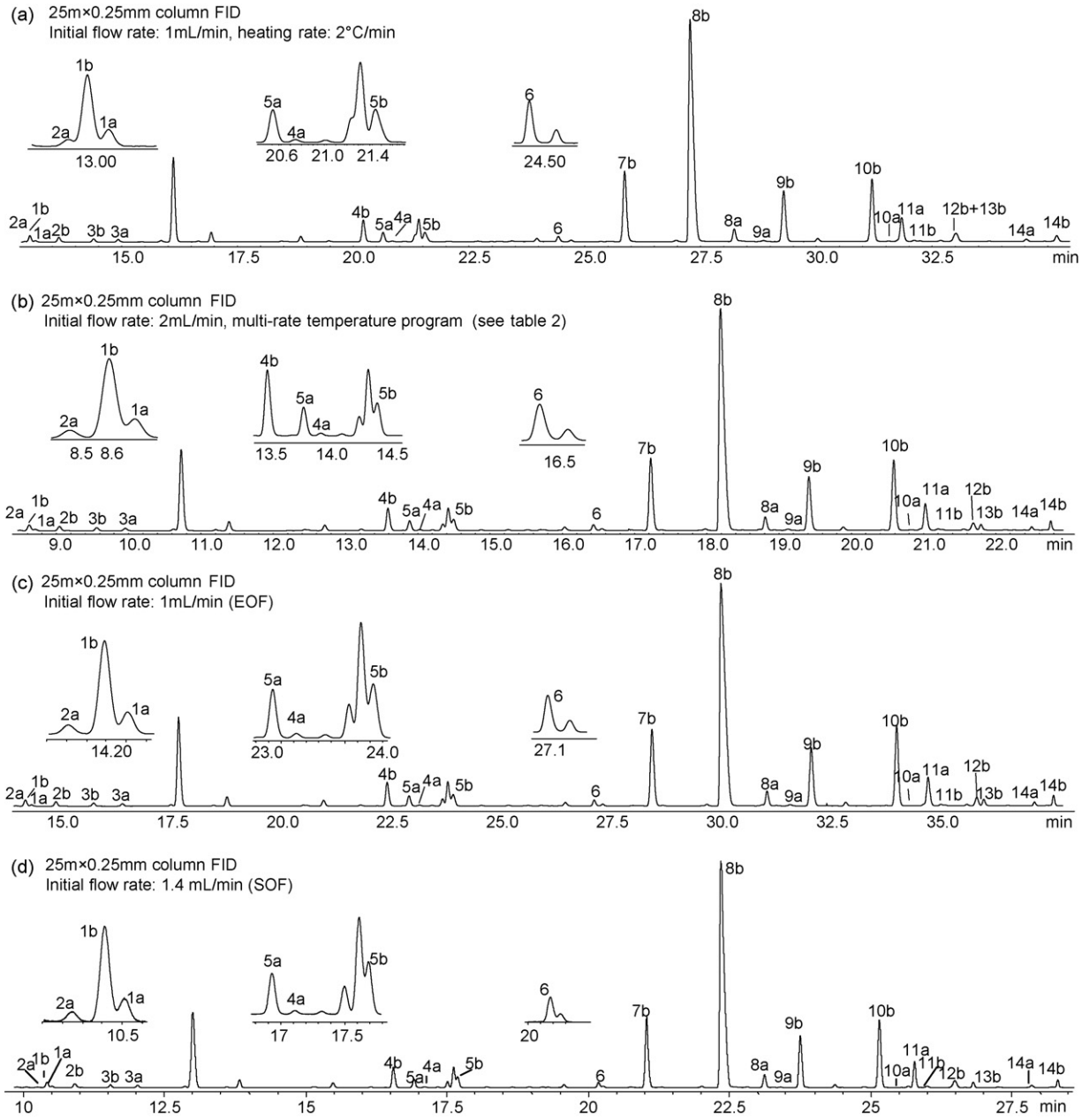

Fig. 1. Es-GC profile of the lavender e.o. analysed under different conditions with the conventional d_c column. For analysis conditions see text and Table 2. Peak identification: (1) α-pinene, (2) camphene, (3) β-pinene, (4) α-phellandrene, (5) limonene, (6) 1-octen-3-ol, (7) camphor, (8) linalool, (9) borneol, (10) linalyl acetate, (11) terpinen-4-ol, (12) lavandulol, (13) α-terpineol, (14) lavandulyl acetate; (a) (*S*)-enantiomer, (b) (*R*)-enantiomer.

d_c (25 m × 0.25 mm × 0.25 μm) column and an (approximately) 11 m narrow-bore (11.13 m × 0.10 mm × 0.10 μm) column. Both columns were from MEGA (Legnano, Italy). Shimadzu equipment was used in the method with conventional column. Agilent equipment was used in the method with narrow-bore column.

GC–FID conditions: temperatures: injector: 220 °C, detector: 230 °C, FID data acquisition rate: 50 Hz. Practically, 10 or 20 Hz would be enough even for our fastest analysis using 11.13 m × 0.10 mm column. 50 Hz was selected prior to beginning of experiments to avoid any issues with peak broadening due to insufficiently high data rate. Injection mode: split; for conventional d_c columns: split ratio: 1:50, injection volume: 1 μl, for narrow-bore columns: split ratio 1:397, injection volume: 0.5 μl. All analyses were carried out with helium as carrier gas in constant pressure mode. The initial flow rates resulting from the applied pressures, rather than the pressures themselves, are reported in text and tables. Temperature programs were from 50 to 220 °C at the rates reported in the text.

GC–MS conditions: temperatures: injector: 220 °C, transfer line: 230 °C, ion source: 200 °C, carrier gas: He, flow control mode: constant pressure. The MS operated in electron impact ionization mode (EI) at 70 eV, scan rate: 4.5 scan/s, mass range: 35–350 *m*/*z* (suitable to cover the full fragmentation pattern of most e.o. components). For injection conditions see GC–FID.

All reported data are the means of three repetitions. The following lavender essential oil components were chosen to evaluate the influence of Es-GC conditions on separation: α- and β-pinene (**1**) and (**3**), camphene (**2**), β-phellandrene (**4**), limonene (**5**), 1-octen-3-ol (**6**), camphor (**7**), linalool (**8**), borneol (**9**), linalyl acetate (**10**), terpinen-4-ol (**11**), lavandulyl acetate, (**12**), lavandulol (**13**) and α-terpineol (**14**).

Table 2
Method parameters (initial flow rates and translated heating rates) and measured parameters (analysis times and resolutions of α-pinene enantiomers).

Column dimensions (detector)	25 m × 0.25 mm (FID)											10 m × 0.1 mm	
												(FID)	(MS)
Initial flow rate (mL/min)	2.0	0.3	0.5	0.7	1.0 (EOF)	1.4 (SOF)	1.7	2.3	2.5	2.8	4.0	0.56 (SOF)	0.56 (SOF)
Temperature program													
Initial temperature (°C)	50	50	50	50	50	50	50	50	50	50	50	50	50
Heating rate1 (°C/min)	2.60	0.58	0.90	1.19	1.57	2.02	2.32	2.86	3.02	3.25	4.08	5.53	5.90
Intermediate temperature1 (°C)	74	74	74	74	74	74	74	74	74	74	74	74	74
Heating rate2 (°C/min)	3.30	0.74	1.14	1.51	2.00	2.57	2.95	3.63	3.83	4.13	5.17	7.04	7.50
Intermediate temperature2 (°C)	115	115	115	115	115	115	115	115	115	115	115	115	115
Heating rate3 (°C/min)	15.00	3.34	5.20	6.87	9.08	11.67	13.40	16.49	17.43	18.77	23.52	31.96	34.10
Final temperature (°C)	220	220	220	220	220	220	220	220	220	220	220	220	220
Final time (min)	2.00	8.98	5.77	4.37	3.30	2.57	2.24	1.82	1.72	1.60	1.27	0.94	0.90
Analysis time (min)	22.76	102.13	65.93	49.82	37.65	29.28	25.49	20.69	19.59	18.19	14.50	10.78	10.09
Resolution of α-pinene	0.96	1.01	1.04	1.06	1.07	1.04	1.00	0.93	0.90	0.88	0.72	1.09	1.10

3. Results and discussion

The main aim of the study was to show that the approach and principles of method translation can successfully be applied to speed up Es-GC analysis of an essential oil with CDs as chiral selector, without interfering with the specific mechanism of enantiomer recognition. The following strategy was employed: (a) optimization of the chromatographic conditions affording the best speed/separation trade-off with a conventional d_c column and (b) translation of the method to a narrow-bore column. Step (b) was first operated with FID as detector and then with MS. As for the previous study [5], a limit of resolution of 1.5 for the enantiomers of each marker was fixed to afford correct ee or er determination. In the following, the term *analysis time* indicates the retention time of the last marker object of this investigation.

3.1. Optimization of Es-GC analysis conditions of lavender e.o. with a conventional 25 m × 0.25 mm column

This part involved three main steps: (a) choice of initial conditions for the optimization process (Section 3.1.1), (b) determination of optimal multi-rate temperature program for a predetermined fixed column pressure (Section 3.1.2), and (c) determination of optimal pressure for the normalized optimal multi-rate temperature program (Section 3.1.3). In all cases, column pneumatic conditions are not expressed in terms of column pressure (which is fixed over the entire analysis), but in terms of initial flow rate (i.e. the flow rate at the beginning of the analysis), for easier comparison with flow conditions (such as 2 mL/min of He in 0.25 mm column of any length) recommended by the GC instrument manufacturer and justified in the literature [8]. Once the optimal initial flow rate of a given carrier gas is defined for a given analysis, it does not change with column length and is proportional to the column inner diameters for all translations [8].

3.1.1. Analysis at different initial flow rates with conventional column at constant temperature rate

The lavender e.o. was first analysed with the conventional d_c column under the temperature and flow conditions applied in routine analysis, i.e. helium flow rate 1 mL/min and 2 °C/min heating rate. Under these conditions the chiral markers were well separated with an analysis time of 35.2 min. Table 1 reports order of elution, retention times (t_R) and resolutions (R_S) of the enantiomers of the chiral markers investigated. Fig. 1a reports the Es-GC pattern of the lavender essential oil investigated, analysed under routine analysis conditions. The applied CD derivative afforded baseline separation of all chiral compounds, with the exception of α-pinene (**1**) enantiomers, which were only partially resolved (R_s around 1 under all conditions applied), and of 1-octen-3-ol (**6**) enantiomers, that were not separated at all, while the (*S*)-enantiomers of camphor (**7**), lavandulol (**13**) and lavandulyl acetate (**12**) were not detectable. Moreover, in this analysis (*R*)-lavandulol (**13b**) and (*R*)-lavandulyl acetate (**12b**) co-eluted.

As the starting point for method optimization, the usual routine analysis conditions adopted in the authors' laboratory were applied except for the initial flow rate, which was doubled to 2 mL/min (Table 1) to reduce the time needed for method development. However, this choice did not affect the final optimal conditions.

3.1.2. Determination of the optimal multi-rate temperature program at a fixed initial flow

The investigated e.o. was then analysed by applying a set of different single-ramp heating rates, namely 2.6, 3.3, 5.0, 7.5, 10, and 15 °C/min (°C/t_M). The results for 2.6, 3.3 and 5.0 °C/min are reported in Table 1; those for 7.5, 10, and 15 °C/min are not reported, because with these rates an increasing number of chiral e.o. components, e.g. α-pinene (**1**), borneol (**9**), linalyl acetate (**10**), terpinen-4-ol (**11**), were not separated, and in addition, some enantiomers of different markers and/or components co-eluted.

The rate 2.6 °C/min gave the most satisfactory separation, with an analysis time of about 25.5 min and at the same time a good separation of all compounds, including the (*R*)-limonene (**5b**)/ocimene, 1-octen-3-ol (**6**)/γ-terpinene and (*S*)-linalool (**8a**)/(*S*)-borneol (**9a**) pairs that were not separated at 2 °C/min. The analysis at 3.3 °C/min took about 21 min, but resulted in a poorer resolution of α-pinene (**1**) enantiomers and in the co-elution of (*R*)-lavandulol (**13b**) and (*R*)-lavandulyl acetate (**12b**). Lastly, at 5 °C/min the analysis time was about 16 min, (*R*)-lavandulol (**13b**) and (*R*)-lavandulyl acetate

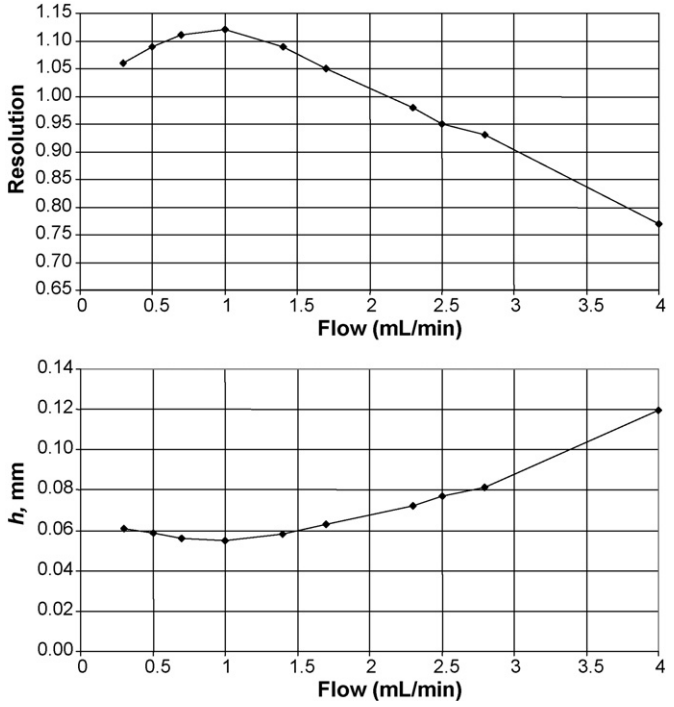

Fig. 2. Diagrams of the variation in resolution (R_s) of α-pinene enantiomers and relative plate heights (h) for α-pinene under different initial flow rates and corresponding multi-rate temperature programs (see Table 2).

(**12b**) were very well separated, but linalyl acetate enantiomers were not discriminated, α-pinene (**1**) enantiomers were separated only very slightly, and (*S*)-camphene (**2a**) and (R)-α-pinene (**1b**) co-eluted.

These experiments showed that, besides separation of the chiral-marker enantiomers, lavender e.o. contains three critical pairs of components: α-pinene (**1**)/camphene (**2**), 1-octen-3-ol (**6**)/γ-terpinene, and (*R*)-lavandulol (**13b**)/(*R*)-lavandulyl acetate (**12b**), whose separations take place at different heating rates (2.6, 3.3 and 2.6 °C/min, respectively). To obtain the best resolution of critical pairs in the shortest time, the following temperature program was planned to be explored. First ramp: 2.6 °C/min from 50 to 74 °C (shortly after elution of α-pinene-camphene group) to obtain the best resolution of 2a-1b-1a analytes; second ramp: 3.3 °C/min from 74 °C till elution of 1-octen-3-ol (**6**)/γ-terpinene-pair; third ramp: 2.6 °C/min after elution of 1-octen-3-ol (**6**)/γ-terpinene-pair till elution of (*R*)-lavandulol (**13b**)/(*R*)-lavandulyl acetate (**12b**)-pair. To find elution temperature of 1-octen-3-ol (**6**)/γ-terpinene-pair, the second ramp was run till the end of the analysis. This dual-ramp program not only provided an acceptable resolution of the 1-octen-3-ol (**6**)/ γ-terpinene-pair, but also resulted in a good resolution of the(*R*)-lavandulol (**13b**)/(*R*)-lavandulyl acetate (**12b**)-pair. The final temperature program consisted of the following ramps from 50 to 74 °C (elution temperature of (*R*)—α pinene (**1b**), retention time 8.62 min) at 2.6 °C/min, then to 115 °C (elution temperature of (*R*)-lavandulol (**13b**), retention time 21.79) at 3.3 °C/min, then to 220 °C at 15 °C/min to clear the column. Fig. 1b reports the Es-GC pattern of the lavender e.o. analysed under the optimized multi-rate temperature program.

3.1.3. Determination of EOF and SOF for multi-rate heating

The next step was to optimize the flow rate by determining the initial EOF (initial flow that maximizes column efficiency and peak resolution) and calculating the initial SOF (initial flow which minimizes analysis time at fixed efficiency) [7].

In any analysis (isothermal or temperature-programmed), optimal flow rate (EOF or SOF) is different for different solutes. As a result, it is impossible to maintain a flow that is optimal for all solutes. A reasonable compromise would be to apply the flow that is optimal for the most critical pair (or the most important pair). Further considerations have to be done in a temperature-programmed analysis. Optimal flow rate for a given solute pair is not a fixed quantity, but declines with the temperature although not as fast as the actual flow declines in a constant pressure mode [16]. As a result there is a mismatch between actual and optimal flow in both constant flow and constant pressure modes. On the other hand, column efficiency is a weak function of flow rate as long as it is reasonably close to optimal. For this reason, it is not worth to use sophisticated flow and temperature programs enabling to achieve perfectly optimal conditions for a given solute pair at every temperature during the analysis. Constant pressure and constant flow modes are both sufficiently effective. In a constant pressure mode, one can therefore speak of optimal *initial* flow rate. This can be EOF that causes the highest column efficiency for a given solute pair and, as a result, its highest resolution (see below). This can also be initial SOF that causes the shortest analysis time for a given resolution of the critical pair. It is important to emphasize that so selected EOF is not necessarily optimal at the conditions at the beginning of the run, rather, it is the value that simply leads to the highest resolution of critical pair. The same considerations can be done for SOF.

Ten different pressures were applied to the column, resulting in different initial flow rates.

The GC method-translator was used to translate the temperature program for each pressure, in order to maintain the same normalized temperature program in all cases. Table 2 reports the initial flow rates, the corresponding translated temperature programs, and the resulting analysis times. These results were used to determine initial EOF (initial flow rate that maximizes column efficiency by minimizing its plate height [8,10]).

In temperature-programmed analysis, H for a peak having standard deviation (σ) and elution retention factor k can be found from the Habgood–Harris formula [17,18]:

$$N = \left(\frac{(1+k)t_M}{\sigma}\right)^2 \quad (1)$$

where N is the plate number corresponding to the peak and t_M is the hold-up time measured at a fixed temperature equal to the peak elution temperature. When N is known, H can be found as follows:

$$H = \frac{L}{N} \quad (2)$$

where L is the column length. Although Eqs. (1) and (2) offer a manageable approach to measuring N, H and initial EOF in a temperature-programmed analysis, a shortcut is possible when the only goal is to find EOF.

Resolution, R_s, of two peaks, 1 and 2, can be expressed as follows:

$$R_s = \frac{t_{R2} - t_{R1}}{2(\sigma_1 + \sigma_2)} = \frac{(1+k_{app2})t_{M2} - (1+k_{app1})t_{M1}}{2(\sigma_1 + \sigma_2)} \quad (3)$$

where

$$k_{app} = \frac{t_R}{t_M} - 1 \quad (4)$$

is the apparent retention factor of a peak (which can be substantially different from the elution retention factor, k, in Eq. (1)). Closely eluting peaks have nearly equal elution temperatures and widths. As a result, $t_{M2} \approx t_{M1}$, $\sigma_2 \approx \sigma_1$. This allows Eq. (3) to be simplified as follows:

$$R_s = \frac{\Delta k_{app} t_M}{4\sigma}, \quad \Delta k_{app} = k_{app2} - k_{app1} \quad (5)$$

Table 3
Retention time (t_R), resolution (R_s) and σ values of the enantiomers of the lavender e.o. chiral markers analysed under different conditions.

Column, initial flow rate		25 m, 1 mL/min (EOF)			25 m, 1.4 mL/min (SOF)			10 m, 0.56 mL/min (SOF)					
								FID			MS		
Compound		t_R	σ	R_s	t_R	σ	R_s	t_R	σ	R_s	t_R	σ	R_s
1a	(*S*)-α-Pinene	14.43	1.91	1.1	11.21	1.54	1.0	4.07	0.48	1.1	3.81	0.47	1.0
1b	(*R*)-α-Pinene	14.29	1.95		11.10	1.56		4.04	0.51		3.78	0.47	
2a	(*S*)-Camphene	14.07	1.96	6.8	10.93	1.64	6.5	3.97	0.53	7.5	3.71	0.55	6.9
2b	(*R*)-Camphene	14.98	2.04		11.64	1.63		4.23	0.53		3.97	0.55	
3a	(*S*)-β-Pinene	16.50	2.11	4.7	12.82	1.67	4.7	4.66	0.54	5.4	4.37	0.48	5.4
3b	(*R*)-β-Pinene	15.84	2.05		12.31	1.60		4.47	0.51		4.19	0.50	
4a	(*S*)-β-Phellandrene	23.24	2.00	5.5	18.06	1.55	5.5	6.61	0.53	6.0	6.19	0.41	6.2
4b	(*R*)-β-Phellandrene	22.51	2.00		17.49	1.55		6.40	0.55		6.00	0.52	
5a	(*S*)-Limonene	23.01	1.99	7.3	17.88	1.55	7.3	6.54	0.52	7.8	6.13	0.47	8.4
5b	(*R*)-Limonene	24.01	2.17		18.66	1.63		6.84	0.60		6.40	0.51	
6	1-Octen-3-ol	27.21	1.87	NR	21.16	1.47	NR	7.79	0.53	NR	7.29	0.46	NR
7b	(*R*)-Camphor	28.53	2.26	1E	22.18	1.71	1E	8.13	0.67	1E	7.62	0.59	1E
8a	(*S*)-Linalool	31.14	1.90	6.0	24.21	1.49	6.0	8.93	0.51	5.6	8.36	0.47	5.8
8b	(*R*)-Linalool	30.09	3.32		23.41	2.45		8.61	1.17		8.07	1.00	
9a	(*S*)-Borneol	31.67	2.47	3.1	24.62	1.82	3.1	9.05	0.73	3.1	8.48	0.82	2.6
9b	(*R*)-Borneol	32.14	2.26		24.99	1.75		9.19	0.64		8.60	0.60	
10a	(*S*)-Linalyl acetate	34.46	1.64	3.0	26.79	1.30	3.0	9.84	0.48	3.1	9.20	0.51	2.7
10b	(*R*)-Linalyl acetate	34.09	2.17		26.50	1.65		9.73	0.61		9.11	0.55	
11a	(*S*)-Terpinen-4-ol	34.80	2.12	2.1	27.06	1.65	2.0	9.98	0.60	2.0	9.34	0.49	2.2
11b	(*R*)-Terpinen-4-ol	35.09	2.03		27.28	1.72		10.06	0.56		9.41	0.52	
12b	(*R*)-Lavandulyl acetate	35.90	2.06	1E	27.91	1.65	1E	10.25	0.53	1E	9.59	0.56	1E
13b	(*R*)-Lavandulol	36.06	1.82	1E	28.04	1.47	1E	10.33	0.44	1E	9.67	0.43	1E
14a	(*S*)-α-Terpineol	37.22	1.45	4.6	28.94	1.15	4.5	10.66	0.36	5.6	9.97	0.35	5.3
14b	(*R*)-α-Terpineol	37.65	1.36		29.28	1.08		10.78	0.32		10.09	0.31	

Notes: All t_R values are in min, all σ values are in seconds. 1E, only one enantiomer detected and NR, not resolved.

which together with Eq. (1) yields[1]:

$$R_s = \frac{\Delta k_{app}}{1+k} \cdot \frac{\sqrt{N}}{4} \quad (6)$$

Solving this equation and Eq. (2) for H, one has:

$$H = \frac{\Delta k_{app}^2 L}{16(1+k)^2 R_s^2} \quad (7)$$

Parameters L, k and Δk_{app} are fixed quantities for all analyses that utilize the same column and that are translations of one another [11]. In view of that, the last formula implies that H is inversely proportional to the square of R_s, i.e.:

$$H \propto \frac{1}{R_s^2} \quad (8)$$

This can also be expressed as follows:

$$h = \frac{1}{R_s^2} \quad (9)$$

where

$$h = \frac{16(1+k)^2}{\Delta k_{app}^2 L} \cdot H \quad (10)$$

can be viewed as relative plate height. Eqs. (8) and (9) show that, as for the flow corresponding to the minimum plate height (H), EOF also corresponds to the minimum relative plate height (h) and to the maximum resolution (R_s) of the target peak pair.

The goal of optimizing the initial flow in this analysis was to obtain the best separation-time trade-off for α-pinene enantiomers, i.e. the most critical pair in the analysis.

Resolutions of α-pinene enantiomers for all initial flow rates tested, F_{init}, are listed in Table 2. Plots of functions $R_s(F_{init})$ and $h(F_{init})$ are shown in Fig. 2. The plots show that the EOF is close to 1 mL/min in combination with 1.57 and 2 °C/min as the first two heating rates (Table 2).

As has been reported [8], SOF can be calculated from EOF as SOF = $\sqrt{2}$ EOF. In our study, in which the initial EOF was 1 mL/min, the initial SOF is therefore 1.4 mL/min, and the corresponding first two heating rates are 2.02 and 2.57 °C/min (Table 2). Under these conditions, analysis time was 29.3 min. Table 3 reports retention times, enantiomer resolution and σ values of chiral markers of the lavender e.o. analysed under the optimal conditions determined. The lavender e.o. profiles at EOF and SOF are shown in Fig. 1c and d, respectively.

3.2. Translation of the method to a narrow-bore column with FID

The optimized SOF method with conventional (25 m × 0.25 mm × 0.25 μm) column was then translated to a narrow-bore (NB) column (11.13 m × 0.1 mm × 0.1 μm) coated with the same stationary phase. Parameters of the translated method are shown in Table 2 and the e.o. profile in Fig. 3a. As part of the method translation, flow rate was reduced in proportion with the column d_c, i.e. from 1.4 to 0.56 mL/min, thus assuring SOF operation of the NB column. Under these conditions, and because both columns had very similar length/d_c ratio, translation did not affect resolution of any peak pair. Table 3 confirms this expectation (taking 10–20% inaccuracy in measurement of peak resolution into account). Table 3 also reports peak retention time and σ values in the translated method. Retention data in Table 3 show that translation reduced analysis time by 2.7 times, without loss in peak resolution.

3.3. Evaluation of MS as detector

The SOF analysis conditions with FID were then translated to the Es-GC–MS of the same lavender e.o. Table 3 reports parameters

[1] In isothermal analysis where $k_{app} = k$, this formula converges to a familiar expression [19] $R_s = (\Delta k/1+k) \cdot (\sqrt{N}/4)$.

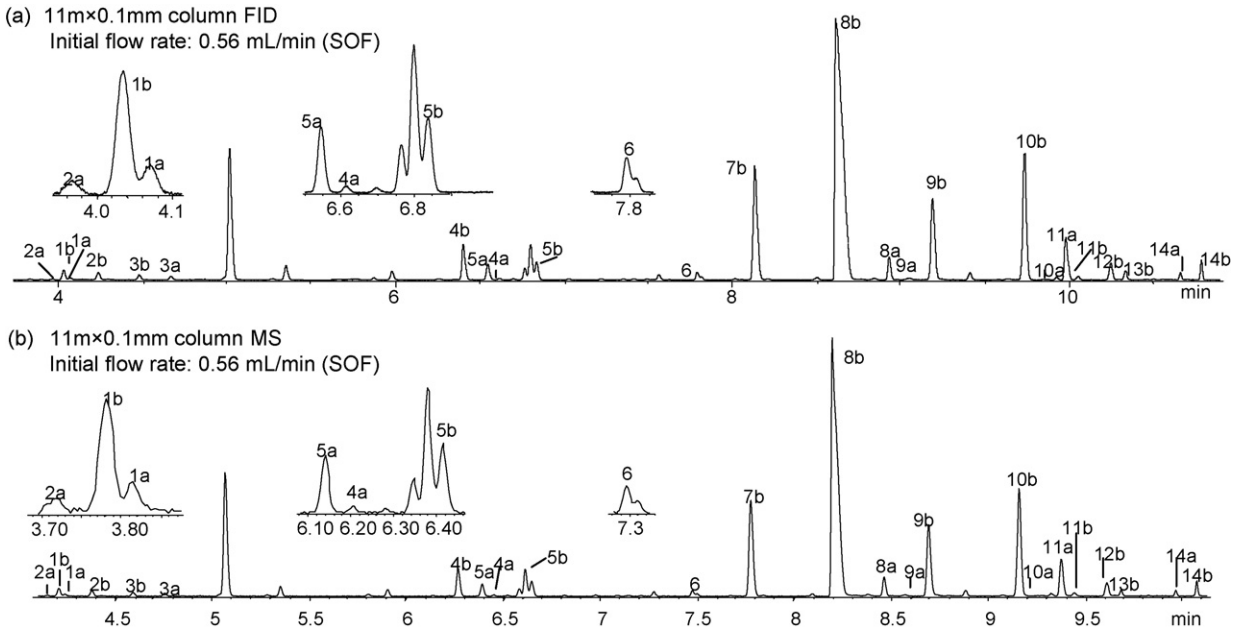

Fig. 3. FID and MS Es-GC profiles of the lavender e.o. analysed under SOF conditions with the narrow-bore column. For analysis conditions see text and Table 2. For peak identification see caption of Fig. 1.

t_R, σ and R_s of the marker components of the e.o. investigated with the NB column using both FID and MS as detectors. The translation further reduced analysis time without losing peak resolution. As is shown in Table 3, translation from the FID method with conventional column at SOF to the MS method with NB column at SOF overall reduced analysis time by 3 times (retention time of the last peak was reduced from 29.28 to 10.09 min).

4. Conclusions

The results show how effective optimization of a Es-GC method and the translation approach can be in reducing analysis time. The use of the optimized Es-GC conditions enabled separation and efficiency to be kept constant in the analysis of a lavender e.o. taken as model, while drastically reducing analysis time from about 37.6 min for the routine method to 29.3 min for the optimized method with conventional d_c column, and to 10.8 (FID) and 10.1 (MS) min with the corresponding NB column; the time required for the whole chromatographic run was reduced from about 87 min for the routine method to 40 min for the optimized method and 15 (FID) or 13.5 (MS) min respectively for the NB columns.

These results also show that Es-GC analysis with CD as chiral selectors can be speeded up, not only by using MS as a further dimension for chiral discrimination [5] but also by effectively tuning the chromatographic conditions with conventional column, and transferring the method to short narrow-bore columns, keeping separation unvaried.

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References

[1] W.A. Koenig, D.H. Hochmuth, J. Chromatogr. Sci. 42 (2004) 402.
[2] C. Bicchi, A. D'Amato, P. Rubiolo, J. Chromatogr. A 843 (1999) 99.
[3] V. Schurig, J. Chromatogr. A 906 (2001) 275 (and reference cited therein).
[4] P.A. Levkin, V. Schurig, J. Chromatogr. A 1184 (2008) 309 (and reference cited therein).
[5] C. Bicchi, E. Liberto, C. Cagliero, C. Cordero, B. Sgorbini, P. Rubiolo, J. Chromatogr. A 1212 (2008) 114.
[6] L.M. Blumberg, J. High Resolut. Chromatogr. 20 (1997) 597.
[7] L.M. Blumberg, J. High Resolut. Chromatogr. 20 (1997) 679.
[8] L.M. Blumberg, J. High Resolut. Chromatogr. 22 (1999) 403.
[9] L.M. Blumberg, M.S. Klee, J. Microcolumn Sep. 12 (2000) 508.
[10] M.S. Klee, L. Blumberg, J. Chromatogr. Sci. 40 (2002) 234.
[11] L.M. Blumberg, M.S. Klee, Anal. Chem. 70 (1998) 3828.
[12] www.chem.agilent.com.
[13] European Pharmacopoeia, 6th ed., 2008.
[14] P.K. Kreiss, A. Mosandl, Flavour Fragr. J. 7 (1992) 187.
[15] C. Bicchi, A. D'Amato, V. Manzin, A. Galli, M. Galli, J. Chromatogr. A 742 (1996) 161.
[16] L.M. Blumberg, W.H. Wilson, M.S. Klee, J. Chromatogr. A 842 (1999) 15.
[17] H.W. Habgood, W.E. Harris, Anal. Chem. 32 (1960) 1206.
[18] W.E. Harris, H.W. Habgood, Programmed Temperature Gas Chromatography, John Wiley & Sons, Inc., New York, 1966.
[19] J.P. Foley, Analyst 116 (1991) 1275.