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Direct resistively heated column gas chromatography (Ultrafast module-GC) for high-speed analysis of essential oils of differing complexities

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Abstract

This study applies Ultrafast module-GC (UFM-GC) with direct resistively heated columns to routine analysis of a group of essential oils of differing complexities (chamomile, peppermint, rosemary and sage). Essential oils were analysed by conventional GC with conventional inner diameter (i.d.) columns (0.25 mm) of different lengths (5 and 25 m long) and by Fast GC and Ultrafast module-GC with narrow bore columns (0.1 mm i.d., 5 m long). Column performance were evaluated and compared through their Grob test, separation number and peak capacity. Ultrafast module-GC was successful in the qualitative and quantitative analysis of essential oils of different compositions with analysis times between 40 s and 2 min versus 20–60 min required by conventional GC. Critical pairs or groups of components were separated by carefully tuning selectivity of the stationary phase to compensate for loss of efficiency due to the use of short columns and high temperature rates. The Ultrafast module-GC results of peppermint e.o. analyses were also validated and compared to those obtained by conventional GC; by measuring precision over time (i.e. repeatability and intermediate precision) and accuracy. Ultrafast module-GC showed a good separation reproducibility affording reliable component identification through the relative retention times and quantitative determination through normalised peak areas. Accuracy data also showed that Ultrafast module-GC and conventional GC normalised areas and areas percentage were perfectly comparable.

Keywords: Direct resistively heated columns; Ultrafast module gas chromatography; Essential oils

1. Introduction

The last 10 years have seen the introduction of electronic pressure control of the mobile phase, high frequency FID detectors and time of flight mass-spectroscopy to detect high-speed peaks, as well as of the software to facilitate method re-validation being necessary when conventional inner diameter (i.d.) columns are replaced by narrow bore columns ([1], and references reported therein). These innnovations have strongly contributed to promoting the use of high-speed GCs for routine analysis [1–3].

In 1998 Blumberg and Klee introduced an objective measure of the speed of a GC analysis [4]. They took the peak width as a measure of analysis speed and defined "... *a fast capillary GC analysis as one with the average peak width of less than* 1 s ...". They also classified approaches involving an average peak width of around 100 ms as *Superfast GC* and those in which it is below 10 ms as *Ultrafast GC*. Starting from these foundations, Magni et al. [5] went into more detail; they defined "Fast GC" as an analysis performed in less than 10 min with columns with i.d. between 0.25 and 0.1 mm, length from 5 to 15 m, temperature programming rates of 20–60 °C/min and peak widths in the range between 0.5 and 2 s. They used the term Ultrafast GC for analyses of 1 min or less, entailing the use of short (2–10 m) narrow-bore columns (0.1–0.05 mm i.d.) and temperature programming rates above 1 °C/s, leading to peak widths of 50–200 ms, as peaks of less than 10 ms are at present difficult to obtain in practice. Table 1 groups the acronyms and abbreviations used henceforth while Table 2 lists the characteristics of the

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Table 1
List of acronyms and abbreviations

Essential oil	e.o.
Inner diameter	i.d.
Conventional GC	C-GC
Conventional inner diameter short column GC	SC-GC
Fast GC	F-GC
Superfast GC	SF-GC
Ultrafast module–GC with direct resistively heated co umns	UFM-GC
25 m long, 0.25 mm i.d. columns	Conventional columns
5 m long, 0.25 mm i.d. columns	Short columns
5 m long, 0.10 mm i.d. columns	Narrow bore columns
Polydimethylsiloxane, 5% phenyl-, 5% vinyl-	SE 54
Polydimethylsiloxane, 7% phenyl-, 7% cyanopropyl-	OV 1701
Polyethyleneglycol	PEG 20M

different GC-speed approaches as classified by Magni et al. [5]. In this article, we adopted the same classification proposed by Magni et al. [5], but, for the sake of clarity, to avoid any confusion due to a not yet fully established terminology, we will indicated the type of high-speed analyses here described with the acronym of the technology we adopted to achieve the applied temperature programming rates i.e. Ultrafast module-GC (UFM-GC). The temperature programming rates of 1–20 °C/s for UFM-GC can only be achieved through direct resistive heating of the capillary columns. Different approaches to direct resistive heating have been described [5,6]. The Ultrafast module (UFM) adopted here was that described by Magni et al. [5] affording temperature programming rates up to 20 °C/s. It derives from the Overton's system [7] for heating very short (1–2 m) narrow bore columns to be installed in a portable Ultrafast GC [8]. This system incorporates heating and temperature sensing elements distributed along the column. In UFM-Trace-GC, the Overton's system has been modified in agreement with Mustacich's patents [6,9–11] extending its use to modules containing capillary columns with a broad range of lengths and diameters and enabling it to be assembled it inside a conventional GC oven. Moreover, the column module can quickly be cooled-down at the end of the analysis (about 1 min from 350 to 40 °C) by activating the oven fan.

Magni et al. [5] demonstrated the reliability and repeatability of UFM-Trace-GC with direct resistively heated columns through standard samples of fatty acid methyl esters, C5–C10 olefin isomers and C10–C25 hydrocarbons.

The main aim of the present article is to evaluate whether UFM-GC can be applied to routine analysis of essential oils. The UFM-GC results with direct resistively heated columns were compared to those of conventional GC (C-GC) with conventional i.d. columns (0.25 mm) of different lengths (5 and 25 m long) and to that of F-GC with narrow bore columns (0.1 mm i.d. and 5 m long) by analysing a series of essential oils of differing complexities (chamomile, peppermint, rosemary and sage). Columns coated with three different stationary phases (SE 54, OV 1701, PEG 20 M) were used and their performances were evaluated and compared through their Grob test (taken as a reference), the separation number (SN, or Trennzahl [12]) and peak capacity, *n*, [13–15] calculated from the Grob test results.

2. Experimental

2.1. Essential oils and Grob test

Chamomile, peppermint, rosemary and sage essential oils were obtained by hydrodistillation through the method described in the European Pharmacopoeia [16]. Grob test kits are available from Sigma Aldrich (Milano, Italy).

2.2. GC analysis

GC analyses were carried out on a ThermoFinnigan Trace GC unit (Rodano, Italy) and a ThermoFinnigan Trace GC provided with the Ultrafast GC option including the UFM-GC column module incorporating a directly resistive heated capillary column granting temperature programming rates up to 20 °C/s. UFM-GC column modules with direct resistive heating were by Thermo Finnigan (Rodano, Italy). Both GC units had high frequency Fast FID detectors (300 Hz, time constant: 6 ms). Data processing was by Chrom-card software (Version 2.01–32 bit) (Thermo Finnigan Rodano, Italy). A series of FSOT-high temperature silylated columns of different length were used. All columns were from MEGA (Legnano, Italy). Table 3 reports the characteristics of the columns used together with the Grob test analysis conditions. Each column was labelled with a Latin number.

2.2.1. e.o. analysis conditions

Unless specified otherwise, the same conditions were applied to the same speed analyses whatever the stationary

Table 2
Characteristic of the different GC approaches in agreement with Magni et al. [5]

	UFM-GC	F-GC	SC-GC	Conventional GC
Column length (m)	2–10	5–15	5	25–30
Column i.d. (mm)	0.10–0.05	0.10–0.25	0.25	0.25–0.32
Analysis time (min)	<1	<10	3–15	10–60
Heating rate (°C/min)	>60	15–60	5–40	1–10
Average peak width (s)	0.05–0.2	0.5–2	1–5	1–10

UFM-GC: Ultrafast module-GC; F-GC: fast GC; SC-GC: conventional inner diameter short column GC.

Table 3
Column characteristics and Grob test conditions and performance

Stationary phase	SE 54					OV 1701					PEG 20M				
GC approach	C-GC	S-GC	F-GC	UFM-GC	UFM-GC	C-GC	S-GC	F-GC	UFM-GC	UFM-GC	C-GC	S-GC	F-GC	UFM-GC	UFM-GC
Column number	I	II	III	IV	IV	V	VI	VII	VIII	VIII	IX	X	XI	XII	XII
Length (m)	25	5	5	5	5	25	5	5	5	5	25	5	5	5	5
Internal diameter (mm)	0.25	0.25	0.1	0.1	0.1	0.25	0.25	0.1	0.1	0.1	0.25	0.25	0.1	0.1	0.1
Film thickness (μm)	0.3	0.3	0.1	0.1	0.1	0.3	0.3	0.1	0.1	0.1	0.3	0.3	0.1	0.1	0.1
Split ratio (SR)	30	30	300	300	300	30	30	300	300	300	30	30	300	300	300
Heating rate (°C/min)	2.1	15	15	150	300	2.1	15	15	150	300	2.1	15	15	150	300
Average linear carrier gas velocity (cm/s)	47.8	50.9	69.3	69.3	69.3	47.8	50.9	69.3	69.3	69.3	47.8	50.9	69.3	69.3	69.3
Void time (s)	59.6	9.8	7.2	7.2	7.2	59.6	9.8	7.2	7.2	7.2	59.6	9.8	7.2	7.2	7.2
Average peak width (W_{av}, s)	4.22	1.31	0.810	0.175	0.118	4.39	1.48	0.827	0.147	0.100	4.76	1.63	0.857	0.147	0.110
Average standard deviation (σ_{av}, s)	1.79	0.552	0.348	0.074	0.050	0.930	0.63	0.351	0.062	0.042	2.02	0.690	0.364	0.062	0.047
Separation number (SN)	39.4	17.1	23.6	12.8	10.1	36.7	14.3	26.9	15.8	11.0	31.7	12.1	24.8	15.3	10.5
Peak capacity (n)	339.5	152.4	235.8	144.5	111.1	261.3	134.1	233.4	165.7	123.6	266.6	112.7	202.1	167.6	117.5

phase coating the column. One microlitre of a solution prepared by diluting 5 mg of e.o. in 1 ml of cyclohexane (1:200) was manually injected into the GC instruments under the following conditions: injection: split, temperature: 230 °C; detector: FID, temperature: 280 °C; carrier gas: hydrogen. Analysis conditions (i.e. split ratio, heating rates and constant flow rates) for each essential oil with each GC speed approach are reported in Table 4.

Characteristic peaks of the e.o. under study were identified by C-GC/MS analysis (scan range 40–300 amu, 8 Hz) and/or standard addition of autentic samples and through their percent area ratio in the e.o. investigated (for UFM-GC runs).

2.3. Method validation

The results of UFM-GC and C-GC analyses of peppermint e.o. were validated by processing the analysis results by: regression analysis, analysis of variance (one-way ANOVA), one sided *F*-test. Precision was evaluated by comparing variance, standard deviation and percentage relative standard deviation (R.S.D.%) between and within data sets. Accuracy was confirmed by comparing corrected target peak areas, percentage total chromatogram peak area and percentage target peak areas of the UFM-GC method to C-GC.

Analyses were carried out by manually injecting 1 μl of the oil diluted 1/200 in cyclohexane containing 0.5 mg/ml of C14 and C19 hydrocarbons as internal standards (ISTD). The linearity in the concentration interval used for the Internal Standard (ISTD) correction was evaluated using standard solutions of C14 and C19.

2.3.1. UFM-GC conditions

Column: PEG 20M; carrier gas: hydrogen, constant flow: 0.8 ml/min; injection mode: split, split ratio: 1/300; injection temperature: 230 °C; FID temperature: 280 °C; block temperature: 250 °C; temperature programme: 50 °C (0.1 min)/500 °C (min^{-1})/250 °C (1 min).

Table 4
Analysis conditions for each essential oil with each GC speed approach

	Conventional GC	SC-GC	F-GC	UFM-GC
Split ratio	30	30	300	300
Chamomile				
Flow rate (ml/min)	1.5	1.5	0.5	0.5 (Col. VIII: 0.7)
Temperature programme	50 °C (1 min)/3 °C (min^{-1})/250 °C Col. V and IX: 100 °C (1 min)/5 °C (min^{-1})/230 °C	50 °C (1 min)/15 °C (min^{-1})/250 °C	(a) 50 °C (0.1 min)/15 °C (min^{-1})/250 °C (b) 50 °C (0.1 min)/30 °C (min^{-1})/250 °C (c) 50 °C (0.1 min)/50 °C (min^{-1})/250 °C	50 °C (0.1 min)/150 °C (min^{-1})/250 °C; (Col. VIII: 50 °C (0.1 min)/500 °C (min^{-1})/250 °C)
Peppermint				
Flow rate (ml/min)	1.5	1.5	0.5	0.5 (Col. XII: 0.8)
Temperature programme	50 °C (1 min)/3 °C (min^{-1})/250 °C	50 °C (1 min)/15 °C (min^{-1})/250 °C	(a) 50 °C (0.1 min)/15 °C (min^{-1})/250 °C (b) 50 °C (0.1 min)/30 °C (min^{-1})/250 °C (c) 50 °C (0.1 min)/50 °C (min^{-1})/250 °C	50 °C (0.1 min)/150 °C (min^{-1})/250 °C; (Col. XII: 50 °C (0.1 min)/500 °C (min^{-1})/250 °C)
Rosemary				
Flow rate (ml/min)	1.5	1.5	0.5	0.5 (Col. VIII: 0.8)
Temperature programme	50 °C (1 min)/3 °C (min^{-1})/250 °C	50 °C (1 min)/15 °C (min^{-1})/250 °C	(a) 50 °C (0.1 min)/15 °C (min^{-1})/250 °C (b) 50 °C (0.1 min)/30 °C (min^{-1})/250 °C (c) 50 °C (0.1 min)/50 °C (min^{-1})/250 °C	50 °C (0.1 min)/150 °C (min^{-1})/250 °C; (Col. VIII: 50 °C (0.1 min)/500 °C (min^{-1})/250 °C)
Sage				
Flow rate (ml/min)	1.5	1.5	0.5	0.5
Temperature programme	50 °C (1 min)/3 °C (min^{-1})/250 °C	50 °C (1 min)/15 °C (min^{-1})/250 °C	(a) 50 °C (0.1 min)/15 °C (min^{-1})/250 °C (b) 50 °C (0.1 min)/30 °C (min^{-1})/250 °C (c) 50 °C (0.1 min)/50 °C (min)/250 °C	50 °C (0.1 min)/150 °C (min^{-1})/250 °C

2.3.2. C-GC conditions

Column: PEG 20M; carrier gas: hydrogen, constant flow: 1.5 ml/min; injection mode: split, split ratio: 1/100; injection temperature: 230 °C; FID temperature: 280 °C; temperature program: 50 °C (1 min)/3 °C (min^{-1})/250 °C.

Characteristic peaks of peppermint e.o., identified by C-GC/MS analysis and standard addition with authentic samples (for UFM-GC), were used as target peaks for the validation procedure. The chromatographic parameters collected for each run were retention time (t_R, min) and peak area.

3. Results and discussion

UFM-GC was here applied to the analysis of essential oils of differing complexities (chamomile, peppermint, rosemary, sage) and the results were compared not only to those of conventional GC (C-GC) but also to those of SC-GC and F-GC. The performance of the columns used for each GC speed approach were evaluated through the Grob test. The UFM-GC analysis results of the peppermint e.o. were also submitted to the validation procedure and the validation results compared to those of GC analyses.

3.1. Column evaluation

The performance of each column was first evaluated through both the results of the Grob test and the separation parameters that can be measured with it.

The parameters currently used to define the metrics of a GC separation are R (resolution of two peaks), SN (the number of well-separated peaks within any homologue pair) and n (the maximum number of peaks that can be separated on a given column) and are calculated with the classical equations:

$$R = \left(\frac{t_{R2} - t_{R1}}{w_b}\right) \quad \text{where} \quad w_b = \frac{w_{b2} - w_{b1}}{2} \tag{1}$$

$$\text{SN} = \frac{t_{R2} - t_{R1}}{2w_h} \tag{2}$$

$$n = \frac{\Delta t}{w_b} \quad \text{where} \quad w_b = 4\sigma \tag{3}$$

where t_{R2} and t_{R1} are the retention times of the two components considered, w_h is the half-height peak width and σ the standard deviation of the peak; w_b in R calculation the average peak width at base-line while in n calculation is four times the standard deviation (σ) of the peak. The Lan and Jorgenson algorithm was here adopted to calculate n because it overcomes some of the limits of the equation previously reported for instance by Giddings and Gruskha [13–15]. These parameters have been considered because they are the most widely used in spite of their limits, in particular their incompatibility and lack of additivity, which were recently discussed in depth by Blumberg and Klee [17].

Grob test and separation parameters were used to evaluate how column performance differed at different GC speeds and whether the loss of column efficiency under UFM-GC conditions is compatible with reliable analyses of essential oils of differing complexities while maintaining the dramatic gain in analysis time with UFM-GC. For each column of the set coated with each stationary phase, E12–E13 separation number (SN), peak capacity (n) [9] and average peak widths of the Grob test components were determined. These data were calculated by applying an initial temperature of 40 °C in combination with heating rates of 2.1 °C/min for the conventional columns, of 15 °C/min for the conventional i.d. short columns and of 15 °C/min for the narrow bore column when used for F-GC and of 150 or 300 °C/min when used for UFM-GC. Hydrogen was used as carrier gas to maximise separation efficiency. Analysis conditions, column characteristics and performance are given in Table 3. For practical reasons we did not apply the rule deriving from the routine experience, by which the efficiency of a short narrow bore column and a conventional column coated with the same stationary phase is the same if the length/i.d. ratio is constant. This was because in general UFM-GC adopts 5 m × 0.1 mm i.d. columns and our aim was to evaluate whether UFM-GC in its conventional configuration could be used for routine analysis of essential oils. Further studies are under way to evaluate the concurrent influence of column length and i.d., heating and flow rates on UFM-GC efficiency and analysis time. We, therefore, expected SN and n to decrease with decreasing column length and with increasing heating rate, although the decrease in efficiency was not directly proportional to the reduction in column length (Table 3). The average peak widths of the Grob test components ranged from 4.76 s for PEG 20 M stationary phase in C-GC to 0.100 s for OV 1701 in UFM-GC in agreement with the values reported by Blumberg and Klee [17] and Magni et al. [5]. In general, column length and inner diameter and temperature rate characterising each GC approach condition column performance while, separation parameters were comparable within each approach independently of the stationary phase. In spite of their short length, narrow bore columns when used in F-GC with analysis conditions calculated by a column translation software [18] showed SN and n higher than in UFM-GC and not far from those of C-GC.

For the analysis of real-world samples using high-speed GC techniques, when the reduction of analysis time is a priority, the price is loss of column efficiency. In spite of this, several high-speed GC applications to real-world samples are successful because efficiency is still sufficient or even higher than required for that given separation. On the other hand, the loss of efficiency due to column shortening can be compensated by adopting stationary phases with an appropriate selectivity to analyse the sample under investigation.

3.2. Analysis of essential oils

Qualitative and quantitative analyses of a series of essential oils of differing complexities were run applying different GC speed approaches; in particular, the separation of

Table 5
Analysis times (AT) and average peak widths (APW) for runs where all target e.o. components were separated expressed as retention times of the last target peak eluted (spiroether for chamomile, β-caryophyllene (SE 54 and OV 1701) and borneol (PEG 20M) for rosemary, sclareol for sage and viridiflorol for peppermint) for each GC speed approach

Essential oil	GC approach	C-GC		S-GC		F-GC (a)		F-GC (b)		F-GC (c)		UFM-GC	
	Stationary phase	AT (min)	APW (s)	AT (min)	APW (s)	AT (min)	APW (s)	AT (min)	APW (s)	AT (min)	APW (s)	AT (min)	APW (s)
Chamomile	SE 54	45.57	2.6	NBS		NBS		NBS		NBS		NBS	
	OV 1701	23.37	3.8	3.66	0.77	9.03	1.1	5.07	0.59	3.35	0.39	0.61 (36.7)	0.082
	PEG 20M	47.48	4.8	12.65		11.20	0.90	6.25	0.56	4.19	0.40	0.93 (55.96)	0.14
Rosemary	SE 54	NBS		NBS		3.29	1.47	2.06	0.94	1.52	0.66	0.85 (51.20)	0.19
	OV 1701	27.32	3.14	NBS		NBS		NBS		NBS		NBS	
	PEG 20M	23.35	3.88	6.11	1.92	4.83	0.73	2.94	0.48	2.04	0.31	0.87 (52.02)	0.15
Sage	SE 54	51.02	3.45	10.98	2.50	9.42	1.04	5.47	0.58	3.60	0.44	1.57 (94.13)	0.19
	OV 1701	57.73	6.55	11.41	0.89	10.51	0.92	4.62	0.57	3.97	0.38	1.59 (95.45)	0.14
	PEG 20M	NBS		NBS		NBS		NBS		4.37	0.40	1.94 (116.6)	0.18
Peppermint	SE 54	NBS		NBS		NBS		NBS		NBS		NBS	
	OV 1701	NBS		NBS		NBS		NBS		NBS		NBS	
	PEG 20M	35.13	3.02	NBS		NBS		3.64	0.49	1.87	0.32	70.41	0.13

NBS: analyses where not all e.o. target components were base-line separated. F-GC heating rates: (a) 15 °C/min, (b) 30 °C/min, (c) 50 °C/min.

critical pairs or groups of components characterising the e.o. will be discussed here. The Grob test indicated that within each type of GC speed approach (i.e. C-GC, SC-GC, F-GC and UFM-GC) columns showed comparable efficiency independent of the stationary phase, the efficiency depending on the GC speed approach selected, therefore, separation of critical pairs or groups was only due to the stationary phase selectivity. Table 5 reports retention times of the last target peak eluted (spiroether for chamomile, β-caryophyllene (SE 54 and OV 1701) and borneol (PEG 20M) for rosemary, sclareol for sage and viridiflorol for peppermint) and average peakwidths for each GC speed approach as an indication of the analysis time for those runs where all target e.o. components were base-line separated.

3.2.1. Chamomile essential oil

Chamomile (*Matricaria chamomilla*) e.o. is characterised by seven sesquiterpenoids, i.e. medium volatility analytes, that are present in different amounts in function of quality and origin. Fig. 1 reports the OV 1701 GC patterns of a chamomile e.o. analysed by C-GC and UFM-GC, together with the list of the characteristic components. This oil is conventionally analysed on apolar stationary phases (OV 1, SE 52 or SE 54) with analysis times above 40 min to obtain

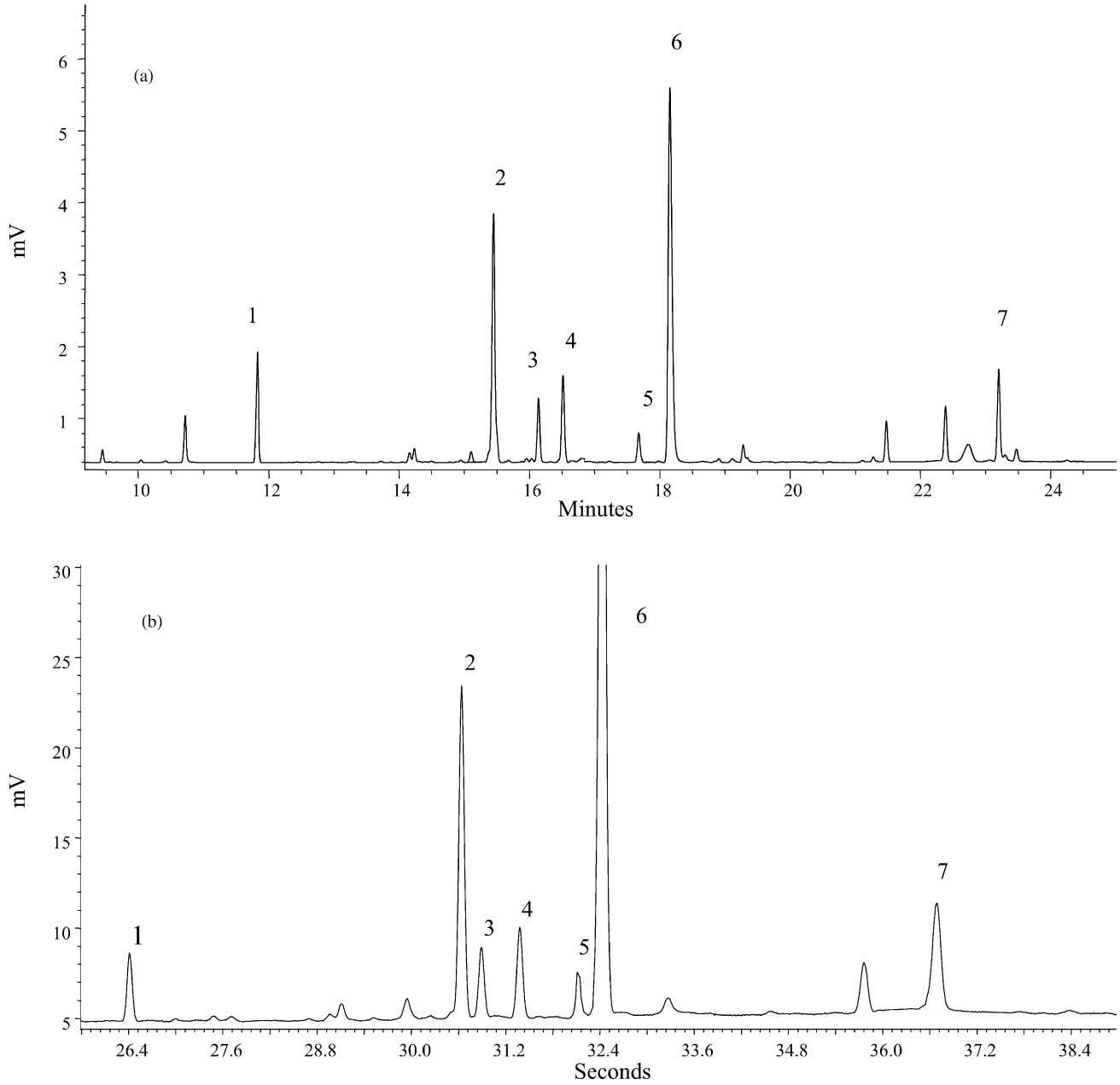

Fig. 1. OV 1701 GC profiles of a chamomile e.o. analysed by C-GC (I) and UFM-GC (VIII). For analysis conditions see text. List of the characteristic components: (1) *trans*-β-farnesene; (2) bisabolol oxide B; (3) α-bisabolol; (4) α-bisabolone oxide A; (5) chamazulene; (6) bisabolol oxide A; (7) spiroether.

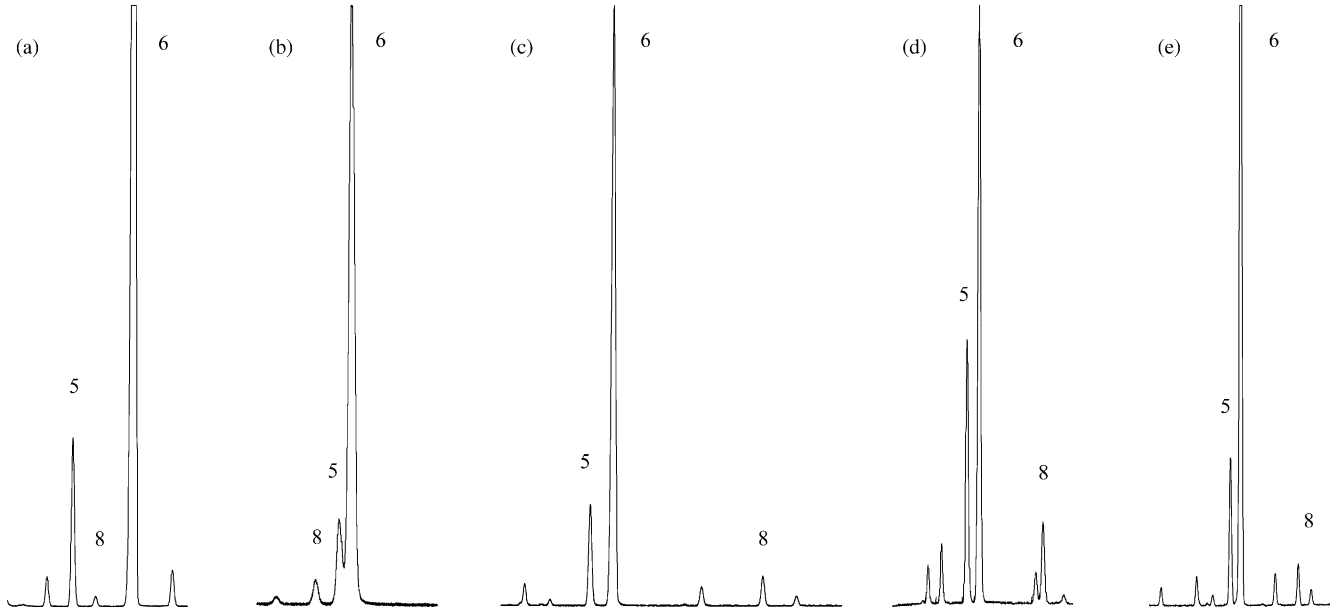

Fig. 2. Parts of the rosemary chromatograms where *p*-cymene (8), limonene (5) and 1,8-cineole (6) elute with OV 1701 (V) (a), SE 54 (I) (b), PEG 20M (IX) C-GC (c), PEG 20M (XI) F-GC at 15°/min (d) and UFM-GC (XII) (e). For peak identification see caption to Fig. 3.

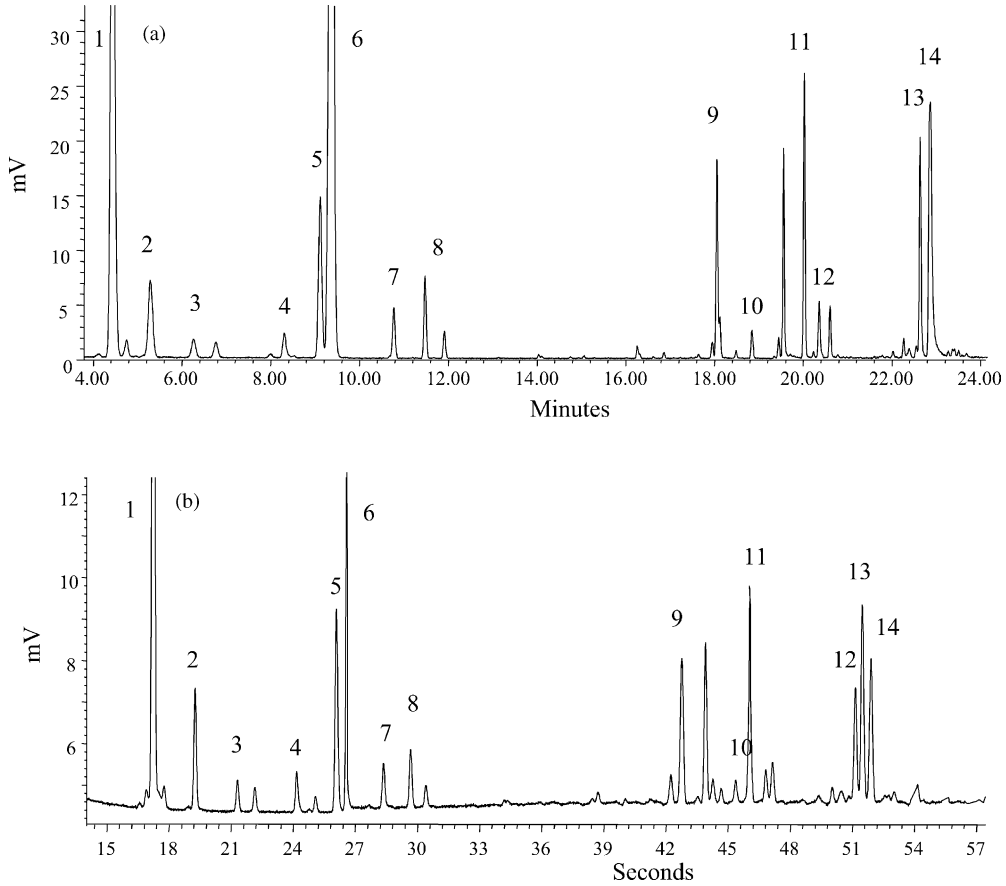

Fig. 3. PEG 20M GC profiles of a rosemary e.o. analysed by C-GC (IX) and UFM-GC (XII). For analysis conditions see text. List of the characteristic components: (1) α-pinene; (2) camphene; (3) β-pinene; (4) myrcene; (5) limonene; (6) 1,8-cineole; (7) α-terpinene; (8) *p*-cimene; (9) camphor; (10) linalool; (11) bornyl acetate; (12) β-caryophyllene; (13) verbenone; (14) borneol.

a base-line separation of all target peaks. The most critical pairs are α-bisabolone oxide A and α-bisabol, i.e. two characteristic components that are base-line separated with C-GC. When operating in SC-GC or F-GC, apolar column efficiencies are not sufficient to separate the critical pairs α-bisabolone oxide A/α-bisabol, therefore, a stationary phase with a better selectivity was necessary. The stationary phase giving α-bisabolone oxide A/α-bisabol base-line separation and at the same time the shortest reduction in analysis time was OV 1701, with which analyses took 4 min for SC-GC and varied from about 9 to 3.5 min depending on the heating rate for F-GC. UFM-GC with OV 1701 column cut the analysis time even more drastically since the base-line separation of all characterising components was achieved in less than 40 s. PEG 20M was as selective as OV 1701 but took longer analysis time, as expected because of its higher polarity. Unlike SC-GC and F-GC, a partial separation of α-bisabolone oxide A and α-bisabol was also achieved with SE 54 in UFM-GC while in F-GC with OV 1701 column their separation improved if the heating rates was increased from 15 to 50 °C/min at the same time of a drastic decrease of analysis time. In both cases the improvement in separation is probably due to the change in selectivity of the stationary phase with temperature [19].

3.2.2. Rosemary essential oil

Rosemary (*Rosmarinus officinalis*) e.o. is a medium complexity oil with composition differing in function of the chemotype considered. The e.o. analysed here belongs to one of the most common chemotypes (1,8-cineole, borneol) and is characterised by about 30 components of differing volatilities ranging from monoterpenoids (i.e. α-pinene) to sesquiterpenoids (i.e. β-caryophyllene). Its analysis by C-GC generally takes about 25 min depending on the GC stationary phase. The most critical components to be separated with an apolar stationary phase are *p*-cymene, limonene and 1,8-cineole in particular when they are present in high amounts, since they eluted over a range of seven retention index units [20,21]. PEG 20M was the most appropriate stationary phase for this analysis because its selectivity was sufficient to separate all target components of this e.o. at the base-line, including *p*-cymene, limonene and

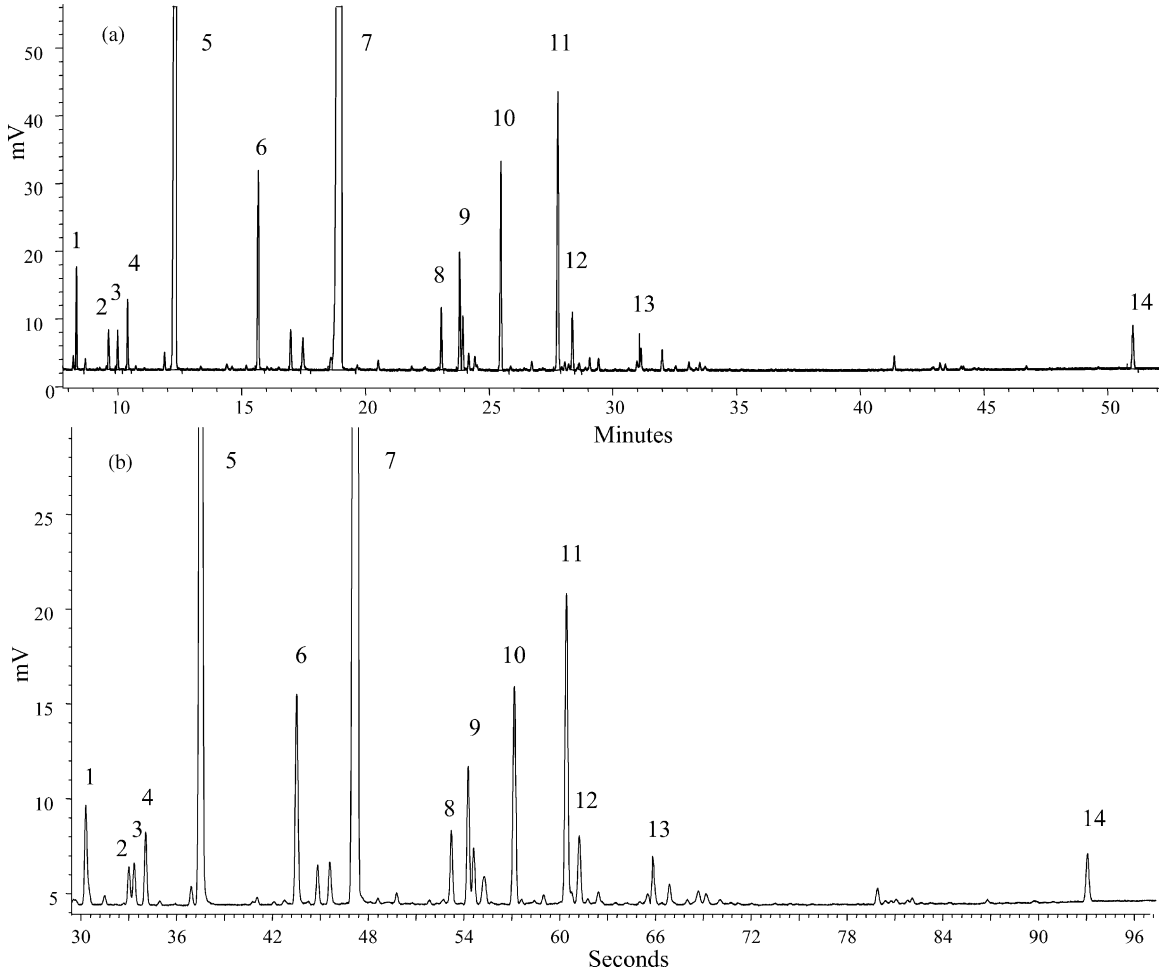

Fig. 4. SE 54 GC profiles of a sage e.o. analysed by C-GC (I) and UFM-GC (IV). For analysis conditions see text. List of the characteristic components: (1) myrcene; (2) *cis*-β-ocimene; (3) limonene; (4) *trans*-β-ocimene; (5) linalool; (6) α-terpineol; (7) linalyl acetate; (8) neryl acetate; (9) geranyl acetate; (10) β-caryophyllene; (11) germacrene D; (12) bicyclogermacrene; (13) geraniol; (14) sclareol.

1,8-cineole with all the GC speed approaches (SC-GC, F-GC and UFM-GC). On the other hand, with SE 54 limonene and 1,8-cineole were separated only partially or not at all, depending on the GC speed approach, while with the exception of C-GC *p*-cymene and 1,8-cineole coeluted with all GC speed approaches with OV 1701 columns. Fig. 2 shows the parts of the SE 54, OV 1701 and PEG 20M C-GC chromatograms and of PEG 20M F-GC and UFM-GC chromatograms where *p*-cymene, limonene and 1,8-cineole elute.

Fig. 3 reports the PEG 20M GC patterns of the rosemary e.o. under study analysed by C-GC and UFM-GC, together with a list of its characteristic components. With PEG 20M, SC-GC analysis time was about 6 min, for F-GC it varied between about 5 and 2 min depending on the heating rates (15, 30 or 50 °C/min), while for UFM-GC it was less than 1 min.

3.2.3. Sage essential oil

Sage (*Salvia sclarea*) e.o. is a medium complexity oil whose composition can vary greatly depending on its origin. The e.o. analysed here belongs to the linalool-linalyl acetate chemotype and is characterised by about 30 components of differing volatilities. This e.o. was considered in this study because for some applications, a minor diterpenoid component (i.e. sclareol) eluting at about 1 h time in C-GC must be quantified. Moreover, with a 25 m PEG 20 M in C-GC the two characterising components, i.e. linalool and linalyl acetate, coeluted, because they eluted over a range of five retention index units [20,21] and were in very high concentrations With the sample investigated, with PEG 20 M linalool and linalyl acetate were only partially separated by C-GC and F-GC at 30 °C/min and coeluted with SC-GC and F-GC at 15 °C/min not only because their retentions with PEG 20M are very similar, but also because samples at high concentration must be analysed to quantify other minor components. With PEG 20M F-GC at 50 °C/min and UFM-GC, a base-line separation of the main components was obtained giving a further evidence on how temperature can influence the selectivity of a stationary phase [19]. SE 54 and OV 1701 have a selectivity suitable to separate all components. With SE 54, C-GC took about 51 min, SC-GC about 11 min, F-GC analysis time ranged from about 10 to 3.5 min depending on the heating rates (15, 30 or 50 °C/min), while for UFM-GC it was less than 2 min. Fig. 4 reports the SE 54 GC patterns of the sage e.o. under study analysed by C-GC and UFM-GC, together with a list of the characteristic components.

3.2.4. Peppermint essential oil

Peppermint (*Mentha piperita*) e.o. is a medium complexity oil characterised by about 30 mono- and sesquiterpenoids. The concentration of some of them is legally limited because of their toxicity. It is generally analysed with a polar stationary phase (PEG 20M) because with apolar stationary phases (SE 54) eight important components (menthone, *i*-menthone, menthofurane, *neo*-menthol, menthol, *neo*-*i*-menthol, β-caryophyllene, *i*-menthol), some of them present in very high percentages (mainly menthone and menthol) eluted over a range of 40 retention index units [20,21]; as a consequence, with apolar column some of them very often coeluted because column efficiency did not compensate the lack of selectivity. OV 1701 behaved similarly to SE 54. With PEG 20M, C-GC analysis time was about 35 min, SC-GC took about 8 min with a partial coelution of *i*-menthone and menthofurane, with F-GC analysis time decreased from 6 to about 2 min depending on the heating rates (15, 30 or 50 °C/min) but at 15 °C/min did *i*-menthone and menthofurane partially overlapped, while analysis time for UFM-GC was about 70 s with all analytes base-line separated. Fig. 5 compares the SE 54 and PEG 20M C-GC patterns of the peppermint characteristic group of peaks and their PEG 20M separations by F-GC and UFM-GC. Fig. 6 reports the PEG 20M patterns of the peppermint e.o. under investigation analysed by C-GC and UFM-GC, together with a list of the characteristic components.

These results show that UFM-GC produces average peak-widths all within the limits given by Magni et al. [5] for Ultrafast GC and that when short analysis time is a priority, and it is achieved by shortening columns and reducing their inner diameters, the loss of efficiency can be compensated by a careful tuning of the selectivity of the stationary phase. In F-GC and UFM-GC temperature plays an extremely important role not only by reducing the peak width but also by influencing the selectivity of the stationary phase. The temperature effect on selectivity can be overlooked in C-GC because of the excess of column efficiency available for most separation but it becomes fundamental in high-speed GC to compensate the lower efficiency of short-narrow bore columns.

3.3. Validation of UFM-GC results

The UFM-GC results of peppermint e.o. were validated and compared to those obtained by C-GC; in particular precision over time (i.e. repeatability and intermediate precision) and accuracy were investigated.

Validation was carried out following the multi-day validation scheme reported in Table 6; time intervals were chosen on the basis of the analysis time (less than 1 min) and sample stability. Characterizing peaks of peppermint e.o. were used as target peaks for the validation procedure. In agreement with the EURACHEM 1998 guidelines [22], precision

Table 6
Multi-day validation scheme of peppermint e.o. analysis

Sample	Week 1		Week 2		
	Day 1	Day 2	Day 3	Day 4	Day 5
ISTD 1 mg/ml	3 times	3 times	3 times	3 times	3 times
ISTD 0.5 mg/ml	3 times	3 times	3 times	3 times	3 times
ISTD 0.1 mg/ml	3 times	3 times	3 times	3 times	3 times
Mint e.o. + ISTD	14 times	14 times	14 times	14 times	14 times

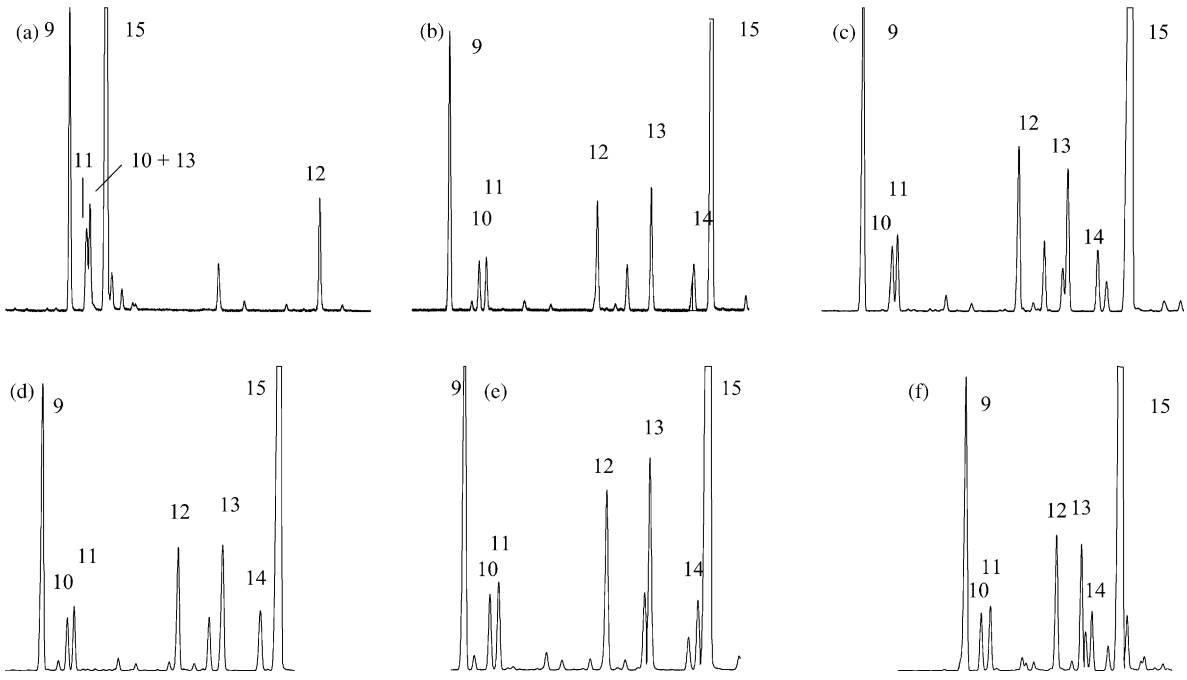

Fig. 5. C-GC patterns of the peppermint characteristic group of peaks by SE 54 (I) (a), PEG 20M (IX) (b), C-GC and their PEG 20M (XI) separations with the F-GC at 15 °C/min (c), 30 °C/min (d), 50 °C/min (e), and UFM-GC (XII) (f). For peak identification see caption to Fig. 6.

over time was evaluated by comparing standard deviation and/or variance between and within data sets.

Table 7 reports the average R.S.D.% on both relative retention times and peak areas of the characterising components of peppermint oil analysed by UFM-GC. Analysis of variance [23] applied to these results showed that characteristic relative retention times and peak areas can be defined. Table 5 also reports accuracy data (normalised areas and percentage areas) of the characterizing components of peppermint oil analysed by both UFM-GC and C-GC. Percentage areas were calculated taking the sum of target peak areas as a reference. The R.S.D.s% for both relative retention times and normalised target peak areas are in full agreement with those reported by David et al. [1] and show a

Table 7
Average R.S.D.% on relative UFM-GC retention times and peak areas and UFM-GC and C-GC normalised areas and percentage areas of the characterizing components of peppermint oil

	Precision		Accuracy			
	R.S.D.% TrRel	R.S.D.% area norm	Area norm		Area (%)	
	UFM-GC	UFM-GC	UFM-GC	C-GC	UFM-GC	C-GC
(1) α-Pinene	0.06	1.17	0.071	0.069	0.96	0.94
(2) β-Pinene	0.09	1.52	0.101	0.103	1.37	1.41
(3) Sabinene	0.07	1.73	0.046	0.047	0.62	0.64
(4) Myrcene	0.10	1.78	0.026	0.029	0.35	0.39
(5) *p*-Cymene	0.09	1.48	0.041	0.043	0.56	0.58
(6) Limonene	0.08	1.36	0.189	0.181	2.58	2.47
(7) 1,8-Cineole	0.08	1.32	0.454	0.445	6.19	6.07
(8) γ-Terpinene	0.06	1.47	0.075	0.069	1.02	0.94
(9) Menthone	0.03	0.68	1.039	1.041	14.18	14.21
(10) Menthofurane	0.02	1.70	0.188	0.179	2.56	2.44
(11) *i*-Menthone	0.02	1.87	0.190	0.188	2.59	2.57
(12) Menthyl acetate	0.03	1.74	0.417	0.421	5.69	5.74
(13) Neomenthol	0.04	1.37	0.431	0.436	5.88	5.95
(14) β-Cariophyllene	0.04	1.51	0.172	0.175	2.34	2.39
(15) Menthol	0.05	0.70	3.463	3.479	47.26	47.49
(16) Pulegone	0.05	1.60	0.189	0.186	2.58	2.54
(17) Germacrene D	0.05	1.31	0.197	0.193	2.69	2.64
(18) Viridiflorol	0.06	1.37	0.041	0.042	0.56	0.58

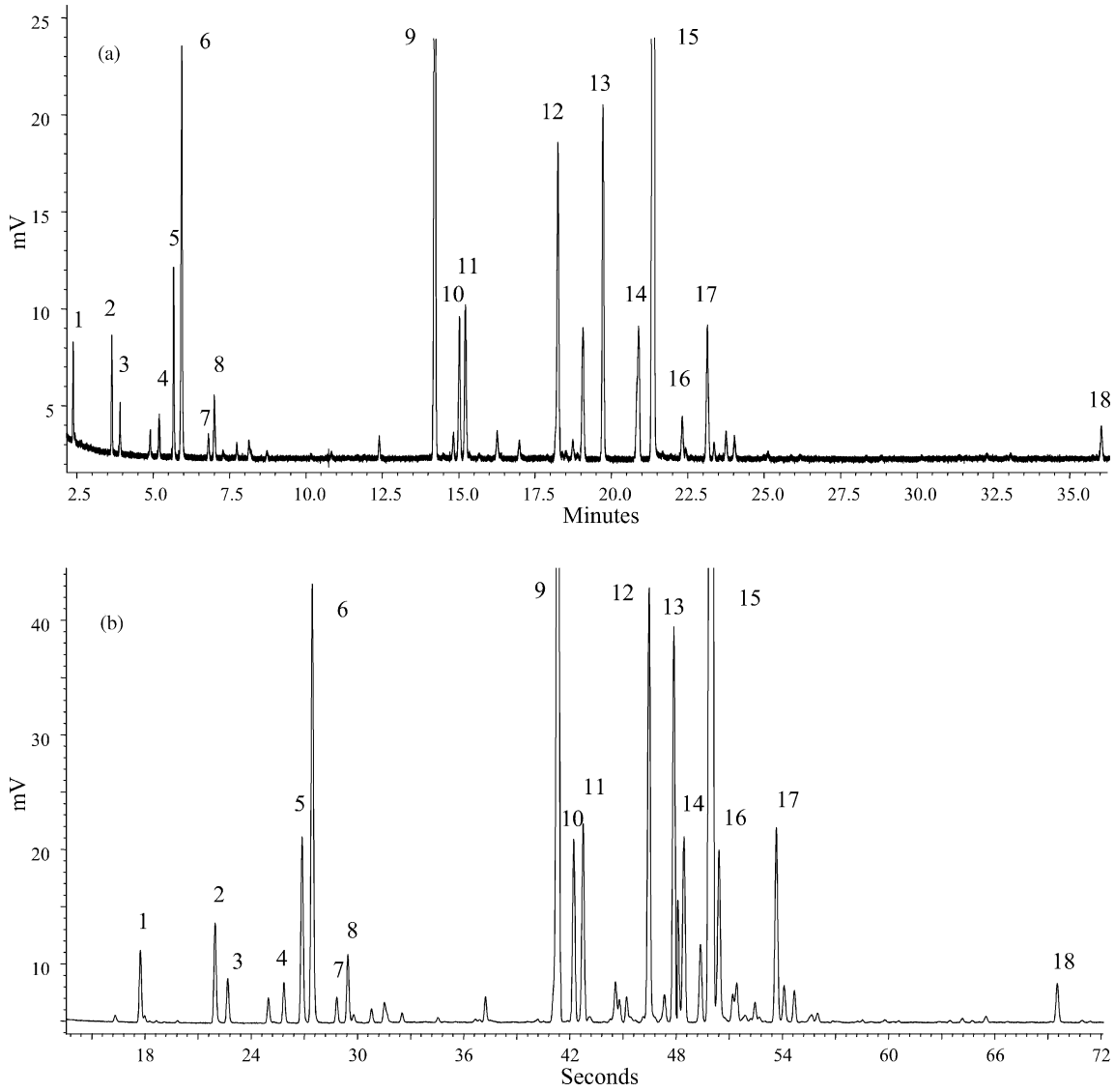

Fig. 6. PEG 20M patterns of a peppermint e.o. analysed by C-GC (IX) and UFM-GC (XII). For analysis conditions see text. List of the characteristic components: (1) α-pinene; (2) β-pinene; (3) sabinene; (4) myrcene; (5) limonene; (6) 1,8-cineole; (7) γ-terpinene; (8) *p*-cymene; (9) menthone; (10) menthofurane; (11) isomenthone; (12) menthyl acetate; (13) neomenthol; (14) β-caryophyllene; (15) menthol; (16) pulegone; (17) germacrene D; (18) viridiflorol.

high precision over time (i.e. repeatability and intermediate precision). It should be stressed that the sample investigated consisted of components of differing volatilities (as for instance α-pinene and viridiflorol) and present in widely differing amounts (as for instance myrcene and menthol). The higher R.S.D.% variations of the more volatile components than the less volatile ones may be explained with the fact that with narrow bore columns irregular sample transfer may occur, because of the very high split ratios required to avoid column overloading [24].

However, these results show that in UFM-GC a good reproducibility can be obtained in terms of separation, and that as a consequence, relative retention times can be a reliable tool for component identification and normalised peak areas can be used for quantitative investigation and for sample characterisation. Accuracy data show that both UFM-GC normalised areas and areas percentage are perfectly comparable to those of C-GC even with manual injection meaning that UFM-GC can be successfully applied to the analysis of peppermint essential oil.

4. Conclusions

This study shows UFM-GC to be successful in the qualitative and quantitative analysis of essential oils of differing compositions. The essential oils investigated are fairly representative of everyday separation problems in this field, since they consist of components with wide ranging volatilities, and are characterised by major and minor components,

separated in C-GC, but co-eluting with the usual stationary phases in high-speed GC or else containing groups of characteristic components of similar chromatographic behaviour. UFM-GC allows us to reduce drastically the analysis time although the very high column heating rates produce changes in selectivity compared to C-GC that are more marked than those of classical F-GC. This change in selectivity may make more difficult to transfer a method from C-GC to UFM-GC than to F-GC making it necessary to resort to a selectivity tuning to obtain baseline separation of critical couples or groups of analytes as it is shown above, and thus requiring the re-validation of the method. In any case, when applied to routine analysis, the reduction of analysis time with UFM-GC is so high to make competitive the method re-validation.

A forthcoming publication will discuss in depth the use of different GC speed approaches to separate the critical pairs or groups of analytes in the essential oils here investigated, together with the influence of narrow bore column lengths, GC conditions and flow rates on separation and on the reproducibility of retention times, [25]. They will be evaluated on the basis of the separation measure, *S*, the universal parameter recently introduced by Blumberg and Klee [17] to evaluate the metric of separation in chromatography.

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