Multidimensional Capillary GC–GC for the Analysis of Real Complex Samples. Part II. Enantiomeric Distribution of Monoterpene Hydrocarbons and Monoterpene Alcohols of Cold-Pressed and Distilled Lime Oils

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Abstract: The enantiomeric distribution of sabinene, β-pinene, limonene, linalool, terpinen-4-ol, and α-terpineol for cold-pressed Key lime oils type A and type B, cold-pressed Persian lime oils, and distilled lime oils has been determined by a fully automated, multidimensional, double-oven (GC–GC) system using a SE-52 precolumn and a β-cyclodextrin main column. This system allows fractions to be multransferred during the same GC analysis and the use of the two GCs independently when the multitransfer option is not used. The results allow us to distinguish cold-pressed Key lime oils type A and type B, cold-pressed Persian lime oils, and distilled lime oils. J Micro Sep 10: 203–212, 1998

Key words: *multidimensional gas chromatography; enantioselective gas chromatography; lime oils; monoterpene hydrocarbons; monoterpene alcohols*

INTRODUCTION

The enantiomeric distribution of the components of essential oils can provide useful information on the genuiness of the oils [1–13], on their quality and on the extraction techniques employed [14–17], on their geographic origin [18, 19], and on their biogenesis [20]. For example, the ratio between (+)- and (−)-limonene has been used to distinguish genuine lemon and mandarin oils from reconstituted ones [3, 4]; the presence of synthetic compounds mixed with genuine gergamot oils [2], with lavender oils [7], and with some petitgrains oils [8] has been detected from the enantiomeric ratios of linalool and linalyl acetate, as has the geographic origin of bitter orange essential oils [19]; the enantiomeric ratio of terpinen-4-ol is related to the extraction technique for mandarin oils [14] while that of α-terpineol is an indicator of the quality of cardamom and *Laurus nobilis* oils [9]; the ratio of (E)-nerolidol indicates the quality of neroli oils [11], as do the ratios of menthol, menthone, and menthyl acetate for mentha [15] and melissa oils [10].

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Gas chromatography with chiral columns coated with derivatized cyclodextrin is the analytical technique most frequently employed for the determination of the enantiomeric ratio of volatile compounds.

Essential oils are usually complex mixtures, so direct gas chromatographic analysis of the enantiomeric ratio of certain components, though sometimes possible [21–28], is usually difficult, requiring great precision in the choice of experimental conditions and of stationary phases in order to avoid overlaps between the peaks of the enantiomers and those of other components [3, 4, 29]. On-line coupled liquid chromatography–gas chromatography (LC–GC) or multidimensional GC systems permit the prefractionation of the sample and the consequent chiral analysis of single components or at least of simpler fractions than the whole sample. In this way problems of peak overlapping are avoided.

Data in the literature are scant on the use of on-line coupled high-performance LC–GC (HPLC–HRGC) systems for the chiral analysis of components of natural complex mixtures [14, 19, 30], while many examples of the use of multidimensional gas chromatography for this type of analysis are

reported [1, 5, 7, 10–13, 16, 18, 25, 27, 31–47]. This last technique uses the high resolution of gas chromatography both in the pre-fractionation stage and in the main analysis.

The LC–GC systems permit the transfer and the subsequent GC analysis of LC fractions that contain compounds of the same polarity, which show the same chromatographic behavior in liquid chromatogrphy, while GC–GC systems allow us to operate transfers of fractions that contain compounds of the same volatility but of different chemical class, if the two columns are placed in either the same GC oven or two different ovens.

The LC–GC systems allow transfers from HPLC to GC of more than one LC fraction. However, each LC fraction subsequent to the first can be transferred and analyzed by GC only after the end of the previous GC run [48]. The GC–GC systems, on the other hand, permit several fractions to be transferred from the precolumn to the analytical column and analyzed in the same GC run; this possibility is enhanced if a cold trap is placed before the inlet of the second column. Moreover, when HPLC is coupled to HRGC, it is very difficult to transfer different amounts of components that belong to the same class, because these compounds have the same chromatographic behavior in HPLC and so are coeluted. In multidimensional GC the precolumn works at high resolution, allowing separation of components of the same chemical class, which show only small differences in volatility. For this reason, different transfer times can be chosen for each component and different small portions of a component can be transferred. In fact, for the determination in a complex matrix (such as an essential oil) of the enantiomeric ratio of two monoterpene hydrocarbons, limonene and β-pinene, present, for example, in a relative percentage of 70 and 10%, the transfer of different portions of the two components from the LC column to the GC column is impossible, if the components are coeluted in LC. If the two components are separated in the precolumn by GC analysis, the amount then transferred of each can be set so that the concentration analyzed on the main column will give well-resolved peaks; for example, 1% of limonene and 10% of β-pinene.

Lime essential oils are obtained from *Citrus aurantifolia* Swingle (Key lime) and *C. latifolia* Tanaka (Persian lime). Distilled lime oil is obtained by reacting the flavor compounds of a mixture of essential oil/juice/crushed fruits in acid medium. It accounts for about 95% of total lime oil production worldwide. The other 5% is made up of cold-pressed Key lime oil, type A, obtained by centrifugation of the oil/juice emulsion produced by passing the whole fruit through a screw-press which crushes the fruits; of cold-pressed Key lime, type B, obtained by rasping the peel to release the oil; and of cold-pressed Persian lime oil, obtained with the same method as for cold-pressed Key lime, type B [49].

The enantiomeric distribution of some components of lime oils has been reported only in three papers by the Mosandl group [18, 35, 50].

In this article the enantiomeric distribution of some monoterpene hydrocarbons (sabinene, β-pinene, limonene) and of some monoterpene alcohols (linalool, terpinen-4-ol, α-terpineol) of different lime oils is reported. The analyses were carried out with an automated multidimensional GC–GC system developed in our laboratory [51], performing five consecutive transfers in the same analysis.

EXPERIMENTAL

The research was carried out on four cold-pressed Key lime oils type A; one cold pressed Key lime oil type B; eleven cold-pressed Persian lime oils; six distilled lime oils. All the samples were analyzed by injecting 1 μl of a 10% (v/v) solution of essential oil in pentane with a split ratio of 1:10.

The multidimensional system used in this study was a developmental model which consisted of two gas chromatographs Shimadzu 17A, a transfer line and two integrators Shimadzu C-R3A. The instrumental setup and the experimental conditions used were as follows (see Figures 1 and 2):

Gas chromatograph 1

- Two split/splitless injectors at 250°C with two manual flow controllers (injectors 1, 2) and a flame ionization detector at 250°C (FID 1).
- A SE-52 capillary column 30 m × 0.32 mm i.d., 0.40–0.45 μm film thickness (Mega, Legnano, Italy); temperature program: 45°C for 6 min, then to 240°C at 2.0°C/min; carrier He.
- A Valco six-ports ($\frac{1}{16}$-in.) two-position UW type valve (valve 1) with a right-angle drive (A3RADN 6WT) (Valco Europe). The valve has a rotor made of fluorocarbon-filled crosslinked polyimide and a port diameter of 0.40 mm and can be operated up to 350°C. Moreover, this valve is designed for use with fused silica columns using a special adapter (Valco FSR.5-5) consisting of a liner which slides over the fused silica tubing and a ferrule which makes up on the liner.
- A digital valve interface (DVI-220) (Valco Europe) connected to EVENT 91 on gas chromatograph 1.
- One-sixteenth-inch removable fused silica adapters (FSR.5-5 and FSR.4-5) (Valco Europe) to connect the valve and the fused silica tubing.

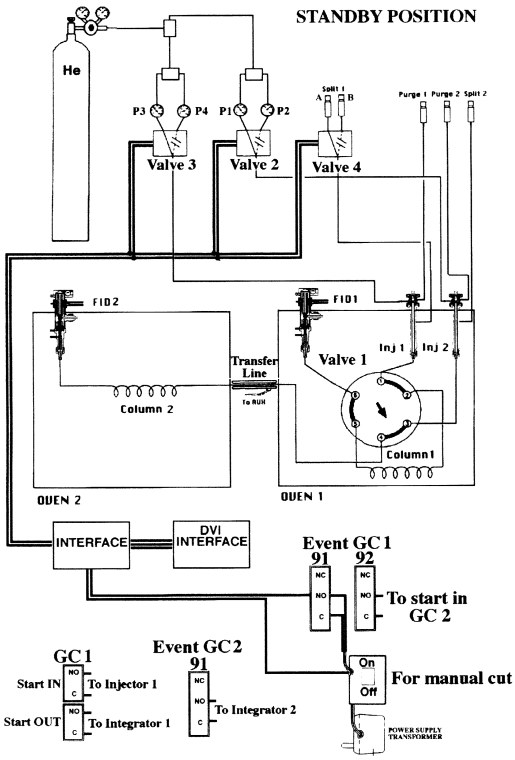

Figure 1. *Pneumatic and electronic scheme of the GC–GC system in the stand-by position.*

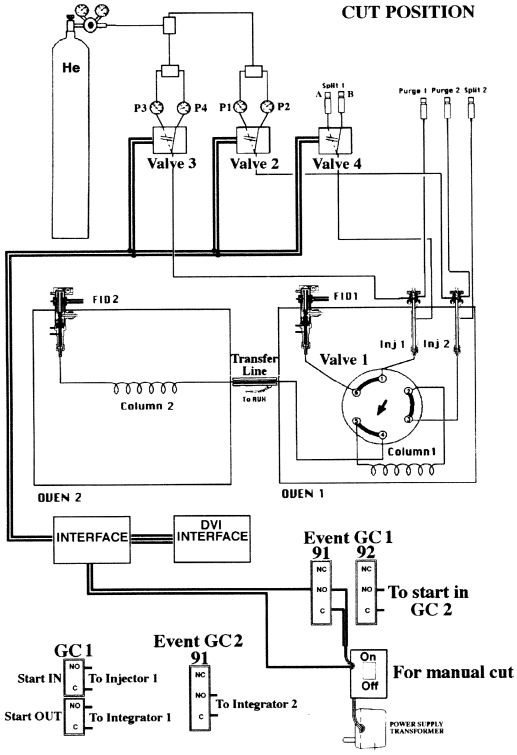

Figure 2. *Pneumatic and electronic scheme of the GC–GC system in the cut position.*

- A solenoid valve (valve 2) to change the carrier pressure (P_1, 110 kPa) (stand-by position, column 2) to higher pressure (P_2, 200 kPa) (cut position, columns 1 and 2) connected to EVENT 92 on gas chromatograph 1.
- A solenoid valve (valve 3) to change the carrier pressure (P_3, 90 kPa) (stand-by position, column 2) to lower pressure (P_4, 2.5 kPa) (cut position, injector 1 and FID 1) connected to EVENT 91 on gas chromatograph 1.
- A solenoid valve (valve 4) which allows the use of two splitter valves (A and B) with different ratios in injector 1.
- An integrator Shimadzu C-R3A connected to start and out signals on gas chromatograph 1.

Transfer line. An aluminum thermoregulated block equipped with a heater assembly and a thermocouple assembly connected to the AUX2 exit on gas chromatograph 1.

Gas chromatograph 2

- A MEGADEXDETTBSβ (diethyl-*tert*-butylsilyl-β-cyclodextrin), 25 m $\times$ 0.25 mm i.d., 0.25 μm film thickness (Mega, Legnano, Italy). Temperature program: 45°C for 6 min, then to 180°C at 2.0°C/min; carrier He; the GC program started with the first cut.
- A flame ionization detector at 250°C (FID 2).
- An integrator Shimadzu C-R3A connected to "start out signal" is an electric event on gas chromatograph 2.

When the six-port valve (valve 1) is in the stand-by position (Figure 1), flow paths are as follows: injector 1 to column 1 to FID 1 and injector 2 through the hot transfer line to column 2 and to FID 2. In this configuration it is possible to carry out analyses independently on the two columns without any change of hardware. Moreover, it is possible to change the carrier pressure and the split ratio for each injector. When valve 1 is switched to the cut position (Figure 2), the flow path is as follows: injector 2 to column 1 through the hot transfer line to column 2 and to FID 2, and at the same time the solenoid valve 2 is switched on to increase the carrier pressure from P_1 (110 kPa) to P_2 (200 kPa).

This ensures that the right retention times are obtained on columnn 1, even for those components eluted after more than one transfer. An increase in pressure is especially important in multitransfer operations because if the same pressure is maintained in the cut position as in the stand-by position, a flow drop occurs when the precolumn (column 1) is added to the main column (column 2). This flow drop causes a shift of the retention times of the components eluted after each transfer, and this does not permit the automatic transfer of more than one fraction during the same analysis. When valve 1 is switched to the cut position, the flow surge may also in some instances extinguish the flame of the detector (FID 1). To prevent this, a solenoid valve (valve 3) is added to decrease the carrier pressure from P_3 (90 kPa) to P_4 (2.5 kPa), thus maintaining a constant flow in the detector FID 1 and protecting it from flow surges due to the absence of column 1 in the flow path. To further regulate the flow at FID 1, another solenoid valve (valve 4) was added. This valve allows splitter 1A to be used in the stand-by position (split ratio for the sample introduction) and splitter 1B in the cut position (high split ratio to rapidly establish pressure P_4). When this multidimensional system operates in the stand-by position, the two columns work independently and so splitters 1A and 2 can be adjusted to optimize the split ratio, while splitter 1B is excluded. When the multidimensional system is used, the splitter of injector 2 is adjusted to allow the increased carrier to escape from the splitter when valve 1 is switched back to the stand-by position and the original pressure P_1 is re-established.

As shown in Figures 1 and 2, the system is completely automated by the use of the external events of the gas chromatrograph. The time at which the valve should be switched to begin the cuts can be determined from a preliminary analysis. After this a fully automated analysis is possible by programming the valve events.

RESULTS AND DISCUSSION

A lime essential oil was first analyzed with the SE-52 precolumn to determine the concentrations of the components of interest and their retention times, maintaining the multidimensional system in the stand-by position. Depending on the retention times and the concentration of each single component, different transfer windows were then chosen and automatically programmed so that well-resolved peaks would be obtained in the chiral column both for components present in the oils at high concentrations and for those present at very low concentrations.

Figure 3 reports the chromatogram of a cold-pressed Key lime oil type B obtained with the SE-52 column and the system in the stand-by position; the chromatogram of the same oil obtained with the SE-52 column and the system in the cut position (on this chromatogram the cuts are shown); and the chromatogram obtained with the chiral column for the fractions transferred from the SE-52 precolumn.

Limonene in all the oils analyzed, β-piene + sabinene in cold-pressed oils, and α-terpineol in distilled oils were only partially transferred because of their high concentrations. β-Pinene + sabinene in distilled oils, α-terpineol in cold-pressed oils, linolool and terpinen-4-ol were quantitatively transferred because they were present in lower amounts.

The percentage content of the transferred components in the analyzed oils and the transfer windows are shown in Table I.

Figures 4–6 show the chiral chromatogram of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, and α-terpineol for a cold-pressed Key lime oil type A, a cold-pressed Persian lime oil, and a distilled lime oil, respectively. Table II reports the enantiomeric distribution in lime oils of the components analyzed.

Cold-pressed Key lime oils type A and cold-pressed Key lime oil type B show practically identical values of the enantiomeric distribution of the analyzed components, except for a small difference in the enantiomeric distribution of limonene: the type B oil shows a (−)-limonene content of 1.8% of the total amount of limonene, while the type A oils show a value (−)-limonene never lower than 2.6%.

Cold-pressed Persian lime oils show the same value for the enantiomeric distribution of limonene as Key lime oils but different values for the enantiomeric distribution of the other components: (+)-β-pinene, (+)-sabinene, (+)-linalool, and (+)-α-terpineol in Persian lime oils represent a higher percentage of the total amount of the component than in Key lime oils, while for terpinen-4-ol the reverse is true. The differences between Persian and Key lime oils can be attributed to the natural characteristics of the two oils and not to the extraction technology. The Key lime oil type B analyzed shows similar values to Key lime oils type A and not to Persian lime oils, although it was obtained with the same extraction technology used for Persian lime oils.

Distilled lime oils, obtained from Key lime fruits, show values of the enantiomeric distribution of the analyzed components different from those shown by the cold-pressed oils, except for β-pinene, which shows the same value as for cold-pressed oils. The other enantiomeric distributions tend to be racemic,

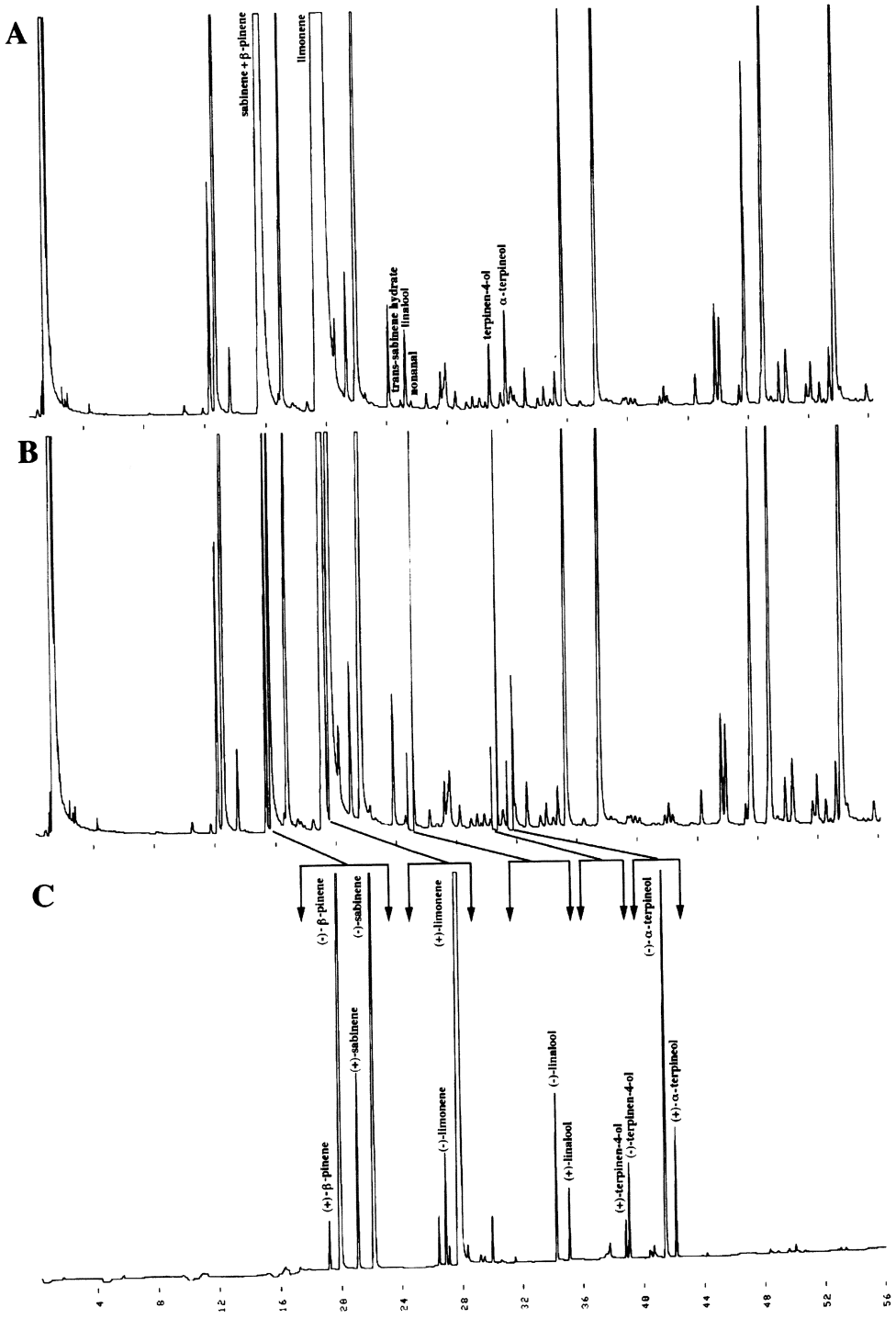

Figure 3. *(a) GC chromatogram of a cold-pressed Key lime oil type B obtained with the SE-52 column. (b) chromatogram of a cold-pressed Key lime oil type B obtained with the SE-52 column with the five heart-cuts. GC–GC chiral chromatogram of the transferred components.*

Table I. *Relative percentage and transfer window of sabinene + β-pinene, limonene, linalool, terpinen-4-ol, and α-terpineol in lime oils.*

	Sample, No	Sabinene + β-pinene	Limonene	Linalool	Terpinen-4-ol	α-Terpineol
Cold-pressed Key lime oils type A	1	21.95	49.28	0.18	0.71	0.35
	2	21.78	49.32	0.18	0.61	0.32
	3	25.45	49.92	0.16	0.37	0.22
	4	24.26	49.39	0.17	0.41	0.24
Cold-pressed Key lime oils type B	5	24.33	49.38	0.17	0.14	0.21
Cold-pressed Persian lime oils	6	13.64	55.24	0.18	0.11	0.30
	7	13.42	56.77	0.18	0.04	0.21
	8	12.41	55.17	0.19	0.08	0.35
	9	12.04	59.81	0.16	0.05	0.20
	10	12.18	59.43	0.17	0.10	0.27
	11	12.28	59.20	0.18	0.08	0.26
	12	12.80	57.62	0.14	0.05	0.20
	13	12.88	58.09	0.15	0.04	0.19
	14	14.10	54.78	0.18	0.07	0.25
	15	13.16	57.36	0.15	0.08	0.25
	16	11.97	59.67	0.16	0.05	0.20
Distilled lime oils	17	1.07	48.32	0.25	0.81	7.59
	18	1.85	49.69	0.13	0.56	8.04
	19	1.19	47.65	0.16	0.79	7.03
	20	0.77	43.54	0.18	0.71	7.00
	21	1.90	49.02	0.16	0.88	7.36
	22	1.23	48.21	0.16	0.82	7.04
Transfer window (min)	—	15.50–15.70	19.55–19.75	24.90–25.35	30.45–30.90	31.50–31.95

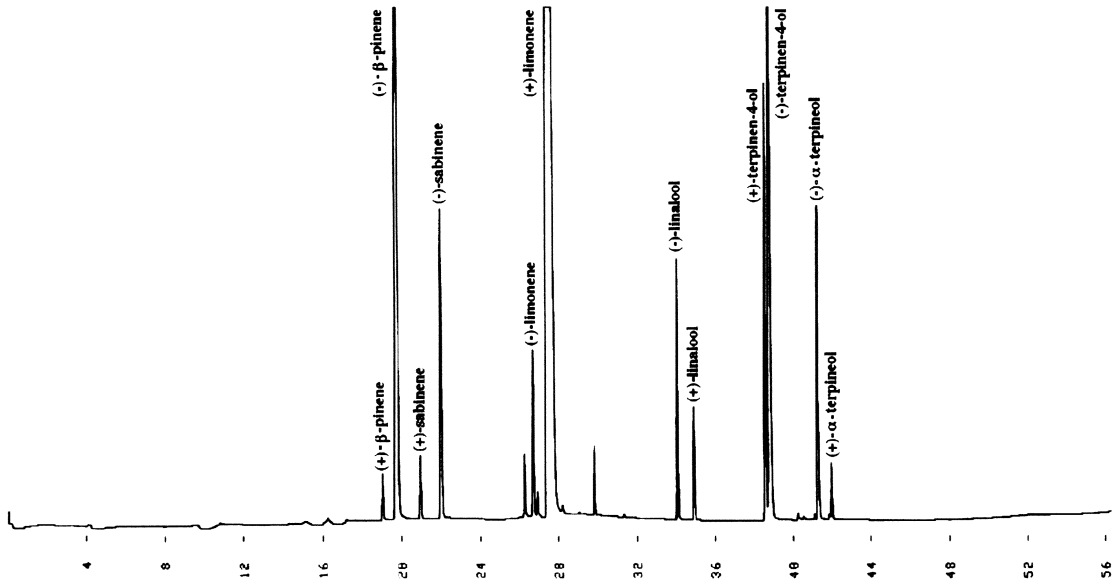

Figure 4. *GC chiral chromatogram of the heart-cut of sabinene + β-pinene, limonene, linalool, terpinen-4-ol, and α-terpineol of a cold-pressed Key lime oil type A.*

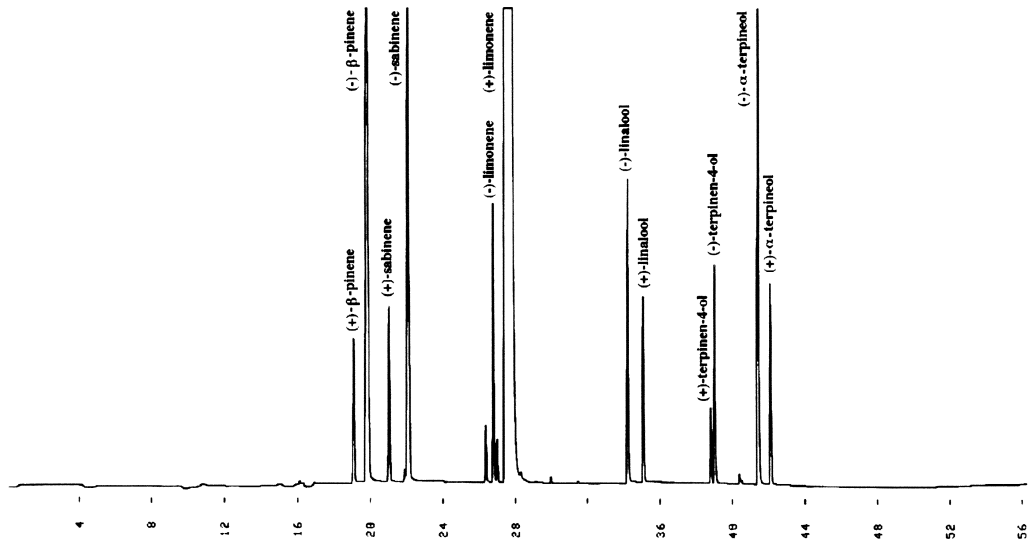

Figure 5. *GC chiral chromatogram of the heart-cut of sabinene + β-pinene, limonene, linalool, terpinen-4-ol, and α-terpineol of a cold-pressed Persian lime oil.*

and linalool shows a racemic composition. This behavior agrees with the chemistry of the distilled lime oils, described in detail by Clark and Chamblee [49].

Results reported in the literature for β-pinene and limonene [18, 35, 50] are for lime oils of different geographical origin; in some cases the samples were laboratory extracted and in other cases the extraction technique is not reported. Values reported for the ratio (+)- and (−)-limonene range from 99/1 to 93/7, while those for (+)- and (−)-β-

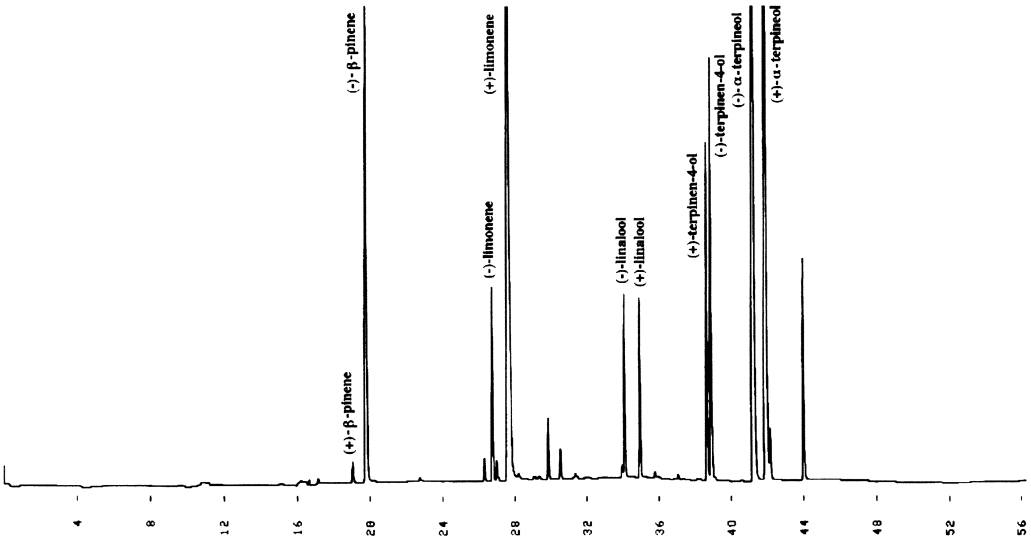

Figure 6. *GC chiral chromatogram of the heart-cut of sabinene + β-pinene, limonene, linalool, terpinen-4-ol, and α-terpineol of a distilled lime oil.*

Table II. *Enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, and α-terpineol in cold-pressed and distilled lime oils.*

Sample, N°	Origin	β-pinene		sabiene		limonene		linalool		terpinen-4-ol		α-terpineol	
		(+)	(−)	(+)	(−)	(−)	(+)	(−)	(+)	(+)	(−)	(−)	(+)
Cold-pressed Key lime oils type A													
1	Mexico	3.5	96.5	15.2	84.8	2.9	97.1	71.5	28.5	29.2	70.8	85.5	14.5
2	Mexico	3.5	96.5	15.2	84.8	2.7	97.3	70.4	29.6	29.5	70.5	85.0	15.0
3	Mexico	3.5	96.5	15.2	84.8	2.8	97.2	70.2	29.8	29.4	70.6	84.0	16.0
4	unknown	3.4	96.6	15.1	84.9	2.6	97.4	70.4	29.6	29.4	70.6	84.0	16.0
Range	—	3.4–3.5	96.6–96.5	15.1–15.2	84.9–84.8	2.6–2.9	97.4–97.1	70.2–71.5	29.8–28.5	29.2–29.5	70.8–70.5	84.0–85.5	16.0–14.5
x	—	3.5	96.5	15.2	84.8	2.7	97.3	70.6	29.4	29.4	70.6	84.6	15.4
Cold-pressed Key lime oils type B													
5	Mexico	3.5	96.5	15.3	84.7	1.8	98.2	70.0	30.0	29.5	70.5	82.8	17.2
Cold-pressed Persian lime oils													
6	Mexico	9.3	90.7	18.7	81.3	0.4	99.6	63.2	36.8	24.9	75.1	75.5	24.5
7	Mexico	9.1	90.9	19.3	80.7	2.4	97.6	61.8	38.2	19.6	80.4	78.3	21.7
8	Brazil	10.2	89.8	23.4	76.6	2.4	97.6	65.0	35.0	20.6	79.4	74.5	25.5
9	Florida	9.7	90.3	18.2	81.8	2.7	97.3	69.3	30.7	22.8	77.2	80.8	19.2
10	unknown	10.2	89.8	20.1	79.9	2.2	97.8	58.5	41.5	23.4	76.6	76.6	23.4
11	unknown	10.2	89.8	20.0	80.0	2.3	97.7	58.5	41.5	23.1	76.9	76.3	23.7
12	Brazil	10.2	89.8	19.3	80.7	2.4	97.6	63.2	36.8	20.0	80.0	76.8	23.2
13	unknown	10.2	89.8	19.2	80.8	2.3	97.7	60.1	39.9	18.6	81.4	75.4	24.6
14	unknown	10.0	90.0	18.4	81.6	2.5	97.5	66.8	33.2	22.0	78.0	79.6	20.4
15	unknown	10.3	89.7	19.2	80.8	2.5	97.5	63.8	36.2	22.7	77.3	77.7	22.3
16	Brazil	10.2	89.8	20.1	79.9	2.1	97.9	54.4	45.6	19.5	80.5	75.2	24.8
Range	—	9.1–10.3	90.9–89.7	18.2–23.4	81.8–76.6	0.4–2.7	99.6–97.3	54.4–69.3	45.6–30.7	18.6–24.9	81.4–75.1	74.5–80.8	25.5–19.2
x	—	10.0	90.0	19.6	80.4	2.2	97.8	62.2	37.8	21.6	78.4	77.0	23.0
Distilled lime oils													
17	Mexico	3.5	96.5	—	—	6.2	93.8	50.0	50.0	43.4	56.6	54.2	45.8
18	Peru	4.0	96.0	—	—	8.7	91.3	49.9	49.9	45.0	55.0	53.3	46.7
19	Mexico	3.5	96.5	—	—	7.0	93.0	49.9	50.1	43.6	56.4	54.4	46.6
20	Mexico	3.2	96.8	—	—	5.5	94.5	49.9	49.9	43.0	57.0	53.9	46.1
21	Ivory Coast	4.0	96.0	—	—	6.3	93.7	49.8	50.2	42.6	57.4	56.8	43.2
22	Mexico	3.8	96.2	—	—	6.0	94.0	49.8	50.2	42.3	57.7	55.8	44.2
Range	—	3.2–4.0	96.8–96.0	—	—	5.5–8.7	94.5–91.3	49.8–50.0	50.2–50.0	42.3–45.0	57.7–55.0	53.3–56.8	46.7–43.2
x	—	3.7	96.3	—	—	6.6	93.4	49.9	50.1	43.3	56.7	54.7	45.3

pinene range from 2/98 to 10/90. These ranges agree with the values reported in this work for the different lime oils.

As can be seen from the chromatograms reported in Figure 3 and from the scheme of the instrument reported in Figures 1 and 2, the system makes it possible to program and carry out fully automated multiple transfers, with reproducibility of the retention times in the precolumn, even for those components eluted after numerous transfers.

The correct use of the system permits the automatic transfer even of those components that elute on the SE-52 column in a critical zone of the chromatogram. For example, linalool elutes column between *trans*-sabinene hydrate and nonanal [Figure 3(*a*)]. It is clear that in such a case the choice of transfer window is critical as imperfect reproducibility of the retention time could case a partial loss of linalool or the presence of *trans*-sabinene hydrate and/or of nonanal in the transferred fraction.

When the system is not used as multidimensional GC, the simultaneous and independent use of the two gas chromatographs is possible without any change to the hardware configuration.

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