Coupling Matters

A Multidimensional HPLC–HRGC System for the Analysis of Real Samples

This article shows the possibility of a hyphenated system consisting of high performance liquid chromatography linked to high resolution gas chromatography (HPLC–HRGC). The potential of this system is demonstrated with complex real samples such as citrus essential oils.

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Introduction

On-line coupling of high performance liquid chromatography to high resolution gas chromatography (HPLC–HRGC) allows high separation efficiency, high speed, simplification of sample preparation and better repeatability.

Complex matrices are not usually analysed directly by a single chromatographic method, even using capillary HRGC. The best approach is to fractionate the sample prior to the gas chromatographic analysis. The simpler mixtures thus obtained may be more homogeneous, and are easier to resolve without the problem of peak overlap.

Off-line methods (such as vacuum distillation, preparative gas chromatography, solvent extraction and classical column liquid chromatography) are laborious, very slow and liable to sample contamination and/or loss at the fraction collection stage (1–3). In comparison with off-line methods, on-line liquid chromatography–gas chromatography (LC–GC) offers some advantages: the amount of sample required is less, no sample work-up is needed, and very complex sample pre-treatment is possible in a fully automated way. In on-line HPLC–HRGC, the sample is first separated by HPLC using a single column, or a combination of columns, to isolate the components of interest. These are then transferred directly to a capillary column in which a further separation is performed using the high efficiency and sensitivity of HRGC. High performance liquid chromatography provides a good pre-separation of samples allowing analysis to be performed by high resolution gas chromatography. It has been used extensively for the pre-separation of complex samples. In addition, different columns and mobile phases can be used to realize the separation of different classes of compounds.

Essentially, there are two methods of transferring fractions from the liquid chromatograph to the gas chromatograph, namely the "concurrent eluent evaporation" (Figure 1) (4) and the "retention gap" (5).

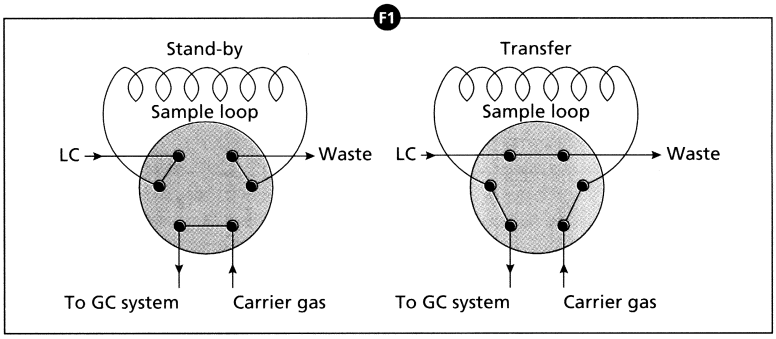

Figure 1: *Loop-type interface. During stand-by, the loop is initially filled by the eluent from the liquid chromatograph. When the fraction of interest is in the loop, the valve is switched (transfer) and the liquid is driven by the carrier gas instead of the HPLC pump.*

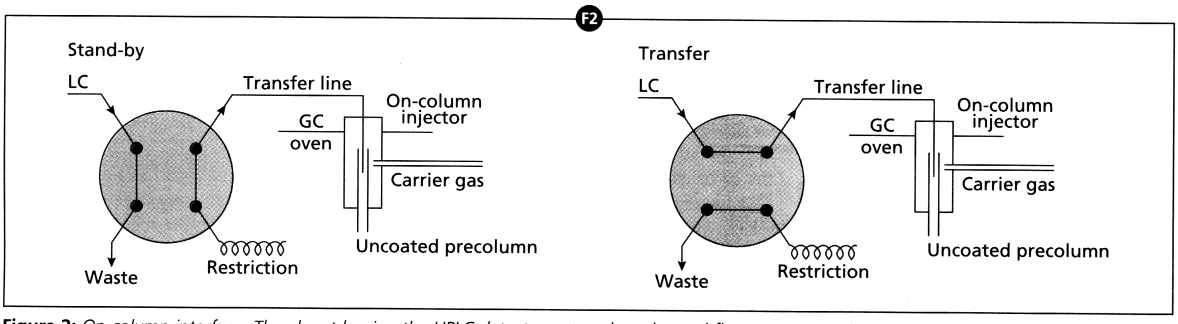

Figure 2: *On-column interface. The eluent leaving the HPLC detector enters the valve and flows to waste when in the stand-by position. When the valve is switched on, the eluent is pumped through the transfer line into the inlet of the on-column injector. At the end of the transfer the valve is switched off, and the eluent again flows to waste.*

Concurrent Eluent Evaporation and the Retention Gap

Concurrent eluent evaporation means complete evaporation of the eluent during its introduction into the gas chromatograph. It allows the analysis of solutes with intermediate-to-high elution temperatures, depending on the volatility of the eluent and the volume of the LC fraction transferred. In this case, the temperature difference between the beginning transfer and the elution of the first sharp peaks is approximately 60–100 °C. In spite of the restrictions concerning elution temperatures, concurrent eluent evaporation is applied for most samples. This technique is preferred to the retention gap technique because of its simplicity, and the possibility of transferring very large LC fractions (1).

The retention gap method (Figure 2) represents the best approach for qualitative and quantitative analysis of samples containing highly volatile compounds. In fact, retention gap allows analysis of substances eluting immediately after the solvent peak, because of the reconcentration of those components by the so-called solvent effects (primarily solvent trapping) (6). However, this method is restricted to fractions of only modest volumes, and the use of a long uncoated precolumn.

Working under conditions that still produce a zone flooded by the eluent (providing solvent trapping), but which cause a large amount of eluent to evaporate during its introduction, we are able to work with a shorter uncoated precolumn or to transfer larger fraction volumes. This method is the so-called "partially concurrent evaporation" in which part of the eluent is evaporated concurrently, that is, during its introduction into the gas chromatograph. The introduction of an early-vapour exit greatly improves partially concurrent evaporation and protects the GC detector.

The volatile fraction of citrus essential oils is a mixture of monoterpene and sesquiterpene hydrocarbons and oxygenated derivatives. The analysis of these oils often requires fractionation of the samples prior to GC analysis (7–16) because of substantial overlap between peaks.

This paper reports the determination of the enantiomeric distribution of linalol and terpinen-4-ol in cold-pressed and distilled lemon and mandarin essential oils.

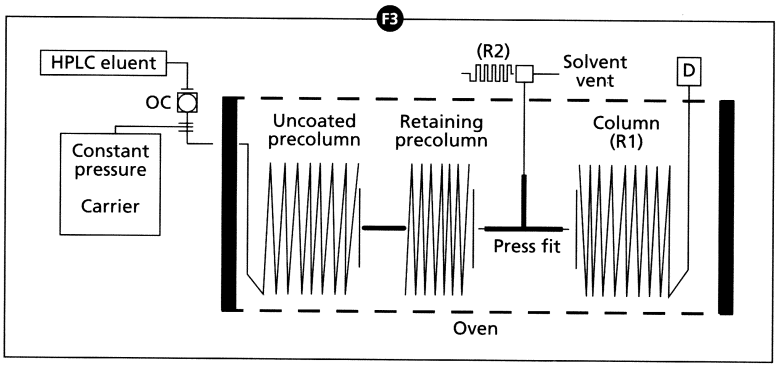

Figure 3: *Schematic of the HPLC–HRGC interface. R1, R2 = restrictors; OC = on-column; D = detector.*

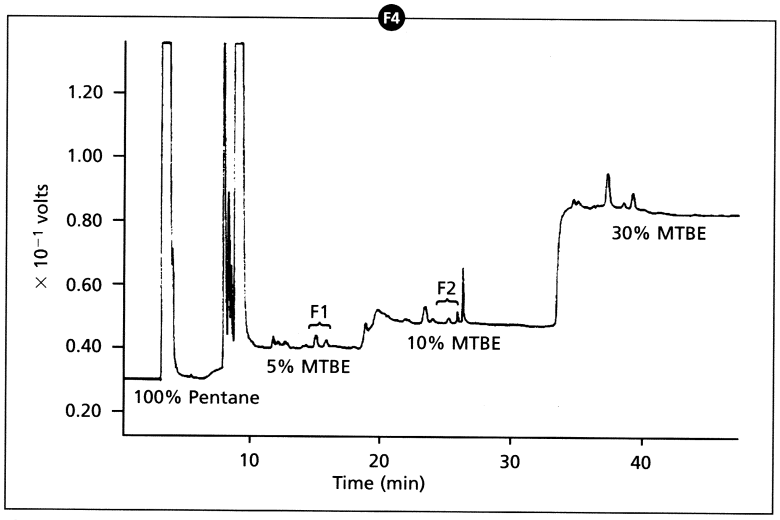

Figure 4: *HPLC chromatogram of lemon oil. F1 = terpinen-4-ol and linalol, F2 = α-terpineol, geraniol and citronellol.*

The determination of the enantiomeric ratio for naturally occurring compounds in essential oils (17) has been proved to be important for odour perception and biological activity. It is known that the odour of enantiomers may be different in quality and activity. The knowledge of the enantiomeric ratio also gives useful information on the source, type and extraction technique of the oil.

A second application shows the pre-separation by LC of a cold-pressed sweet orange oil of which the three aldehydic fractions were transferred to the gas chromatograph and determined without any interferences. The determination of this class of compounds may provide parameters for evaluating the quality of sweet orange oils. Moreover, the qualitative and quantitative determination of the aldehyde content in the oil could give information on the origin and ripeness of the fruit processed.

Experimental

Cold-pressed lemon, mandarin and sweet orange oils were obtained from a Sicilian company (Baller, Messina, Italy); pentane and tert-butylmethyl ether were HPLC grade (Carlo Erba, Milan, Italy).

All analyses were performed using a Dualchrom 3000 instrument (CE Instruments, Milan, Italy). The instrument was configured to use an on-column interface enabling partially concurrent solvent evaporation with an early solvent vapour exit system to reduce the time needed to evaporate the mobile phase. A schematic diagram of the LC–GC interface is shown in Figure 3. A ten-port valve enables cleaning of the column by periodic back-flushing. Analysis was performed under computer control throughout. Step gradient elution is used to separate the fractions corresponding to the interested compounds, which are then transferred to the gas chromatograph.

F5

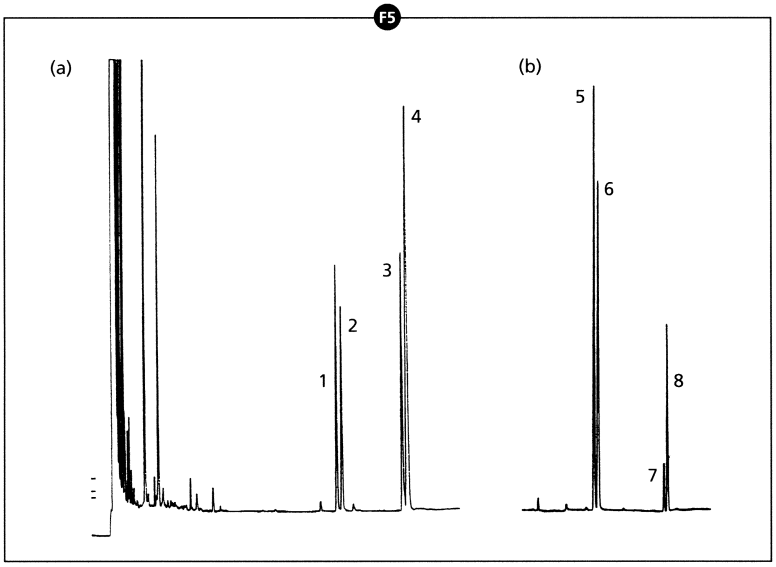

Figure 5: *HPLC–HRGC chromatograms showing the enantiomeric distribution of (a) distilled and (b) cold-pressed lemon oils. Peaks: 1 = (–)-linalol, 2 = (+)-linalol, 3 = (+)-terpinen-4-ol, 4 = (–)-terpinen-4-ol, 5 = (–)-linalol, 6 = (+)-linalol, 7 = (+)-terpinen-4-ol, 8 = (–)-terpinen-4-ol.*

F6

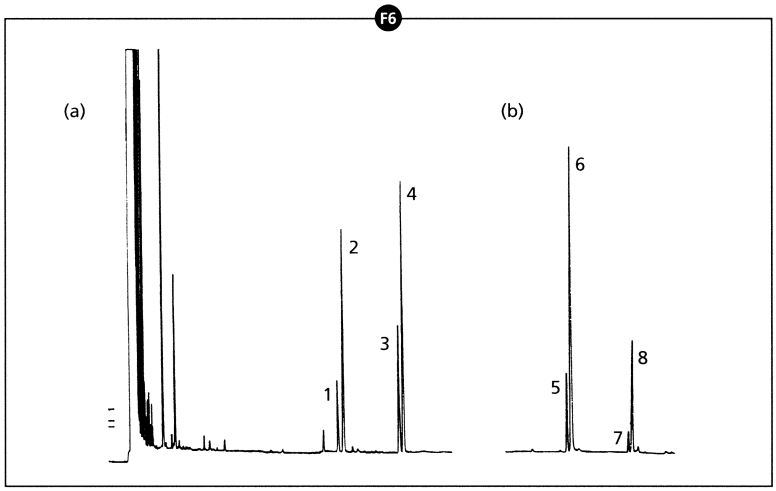

Figure 6: *HPLC–HRGC chromatograms showing the enantiomeric distribution of (a) distilled and (b) cold-pressed mandarin oils. Peaks: 1 = (–)-linalol, 2 = (+)-linalol, 3 = (+)-terpinen-4-ol, 4 = (–)-terpinen-4-ol, 5 = (–)-linalol, 6 = (+)-linalol, 7 = (+)-terpinen-4-ol, 8 = (–)-terpinen-4-ol.*

Monoterpene Alcohols of Lemon and Mandarin Essential Oils

The chromatographic conditions for HPLC pre-separation were: injections of 20 µL of essential oils solution (0.3–1% v/v in pentane) into a 25 cm × 2 mm i.d. column packed with Spherisorb 5 µm (Stagroma); mobile-phase eluent A, pentane; eluent B, tert-butylmethyl ether. High performance liquid chromatography analyses were performed using eluent A for 3 min, then 95:5 eluent A:B for 12 min, then 90:10 eluent A:B for 15 min, then 70:30 eluent A:B for 20 min; flow 200 µL/min; detection was by Micro UVis at 220 nm × 0.5 AUFS. The retention window (from 16 to 19 min, 600 µL) was determined by standard solution of linalol and terpinen-4-ol.

The GC system was composed of the following parts: 10 m × 0.53 mm i.d. fused silica uncoated precolumn (retention gap), deactivated by phenyldimethyl silylation; "retaining pre-column", consisting of a 4 m × 0.32 mm i.d. of fused silica capillary column coated with SE-52 (film thickness 0.45 µm) (MEGA, Legnano, Italy) connected to the retention gap by a press-fit connection; main column, capillary fused silica 25 m × 0.32 mm i.d. coated with 2,3-dimethyl-6-pentyl-cyclodextrin 30% and OV-1701 70% (film thickness 0.25 µm); butt connector with purge line fitted with flow-control valve that automatically switches from high-purging flow during transfer to low-purging flow during analysis.

The temperature during transfer of the LC fraction (3 min) was kept at 45 °C and later increased to 150 °C at a rate of 1 °C/min. Carrier gas (H_2) was delivered at a constant pressure of 120 kPa.

The eluent evaporation rate (155 µL/min) was determined as described elsewhere using hydrogen as carrier gas and was lit at the end of the vapour exit. The time taken

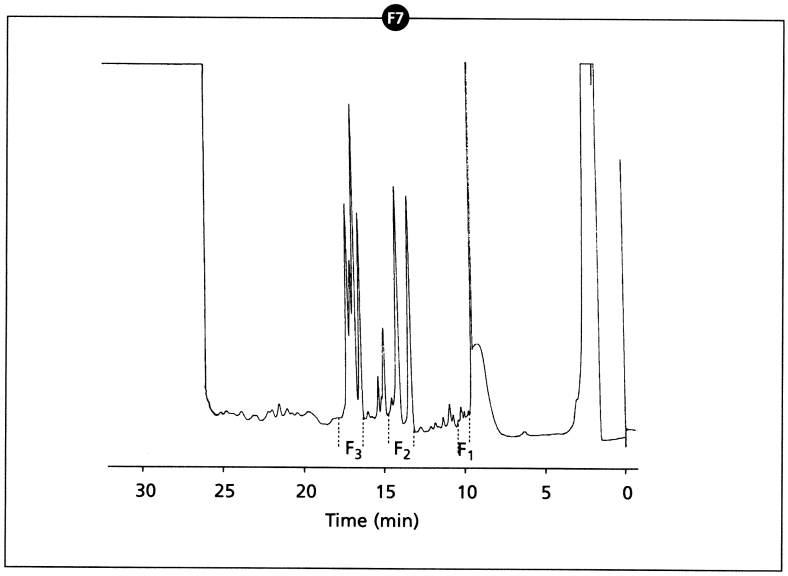

Figure 7: *HPLC pre-separation of sweet orange oil separated the aliphatic, monoterpene and sesquiterpene aldehydes into three fractions (F1, F2 and F3).*

to turn the flame yellow by the evaporating organic eluent gives an exact measurement of its evaporation time.

The solvent vapour exit was switched to low flow 50 s after the end of GC transfer time.

Aliphatic and Terpene Aldehydes of Sweet Orange Oil

A solution of the essential oil in pentane (1%, v/v, 20 µL) was injected into a 10 cm × 2 mm i.d. column packed with Spherisorb 5 µm (Stagroma). Chromatography was started with pentane as mobile phase for 2 min; the composition of the mobile phase was then changed to pentane–ether, 97:3, for 9 min, then 90:10 for 5 min, then 85:15 for 5 min; the column was then backflushed with diethyl ether (1 mL). The mobile phase flow-rate was 180 µL/min. Detection was by Micro UVis at 220 nm × 0.5 AUFS. The retention window was determined using standard substances.

Gas chromatography was performed on a 25 m × 0.32 mm i.d. fused silica capillary

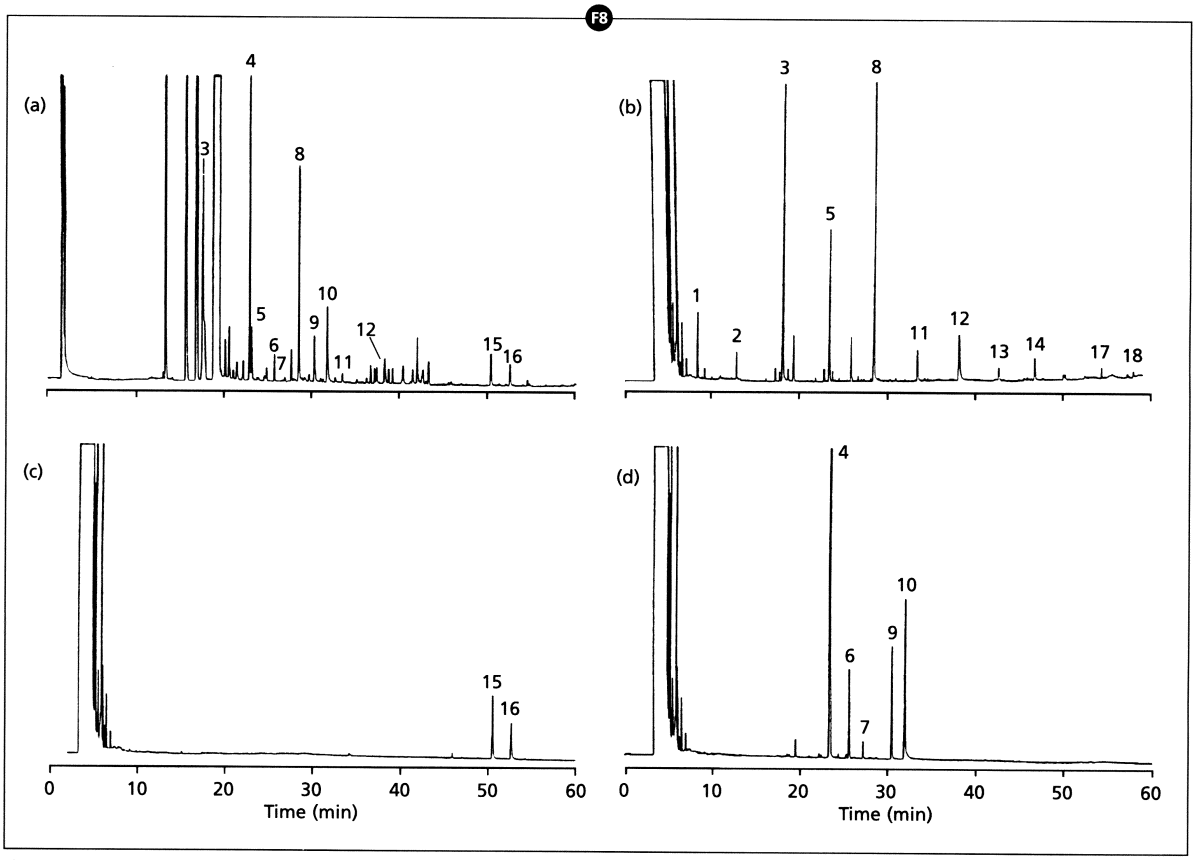

Figure 8: *HRGC chromatograms of (a) sweet orange oil and the fractions from its HPLC separation shown in Figure 7:(b) aliphatic aldehydes (fraction F1); (c) sesquiterpene aldehydes (fraction F2); (d) monoterpene aldehydes (fraction F3): Peaks: 1 = hexanal, 2 = heptanal, 3 = octanal, 4 = linalol, 5 = nonanal, 6 = citronellal, 7 = terpinen-4-ol, 8 = decanal, 9 = neral, 10 = geranial, 11 = undecanal, 12 = dodecanal, 13 = tridecanal, 14 = tetradecanal, 15 = β-sinensal, 16 = α-sinensal, 17 = hexadecanal, 18 = heptadecanal.*

column coated with a 0.40–0.45 µm film of SE-52. The column inlet was connected, by means of a butt connector with purge line, to a 'retaining precolumn' comprising 4 m of the separation column; the pre-column was connected, by means of a press-fit connector (MEGA) to a 10 m × 0.53 mm i.d. uncoated fused silica pre-column (retention gap), deactivated by phenyldimethyl silylation.

The column oven temperature was maintained at 45 °C for 6 min during transfer of the LC fraction, and then increased to 220 °C at 3 °C/min; detection was by flame ionization. The carrier gas (He) was delivered at a constant pressure of 120 kPa.

Table 1: Enantiomeric Ratios of Linalol and Terpinen-4-ol for Samples of Cold-Pressed (n = 24) and Distilled (n = 10) Lemon Oils.

	Cold-Pressed Oils			Distilled Oils		
	X	s	range	X	s	range
(−)-linalol	54	2.4	51–58	53	2.0	51–56
(+)-linalol	46	—	49–42	47	—	49–44
(+)-terpinen-4-ol	20	1.3	18–22	28	1.3	27–21
(−)-terpinen-4-ol	80	—	82–78	72	—	73–79

Table 2: Enantiomeric Ratios of Linalol and Terpinen-4-ol for Samples of Cold-Pressed (n = 15) and Distilled (n = 10) Mandarin Oils.

	Cold-Pressed Oils			Distilled Oils		
	X	s	range	X	s	range
(−)-linalol	17	0.7	16–18	17	1.7	16–20
(+)-linalol	83	—	84–82	83	—	84–80
(+)-terpinen-4-ol	13	0.8	12–14	27	1.9	25–30
(−)-terpinen-4-ol	87	—	88–86	73	—	75–70

Results

Tables 1 and 2 show the enantiomeric distribution of linalol and terpinen-4-ol for mandarin and lemon oils. These tables also show the minimum and maximum values (range), the average values (X) and the standard deviation (s).

Figure 4 shows the HPLC chromatogram of a lemon oil in which the peaks relative to the monoterpene alcohols are identified. Figures 5 and 6 show the HRGC chromatograms of the HPLC-transferred fraction containing linalol and terpinen-4-ol of cold-pressed and distilled lemon and mandarin oils, respectively. As can be seen from the chromatograms in Figures 5 and 6 the HPLC fraction analysed contains only linalol and terpinen-4-ol, so it is possible to evaluate exactly the enantiomeric ratios of these alcohols without interferences. The differences in terpinen-4-ol enantiomeric ratios (see Tables 1 and 2) allow a clear distinction between the more valuable cold-pressed oils and the distilled ones.

The HPLC pre-separation of sweet orange oil under the specified experimental conditions enables the separation of aliphatic, monoterpene and sesquiterpene aldehydes into three fractions marked F1, F2 and F3 (Figure 7). In Figure 8, the gas chromatograms of these fractions are shown (8(b), 8(c) and 8(d)) and compared with the whole oil chromatogram (8(a)). Fractions F1 and F2 contain only aliphatic and sesquiterpene aldehydes, respectively. Fraction F3 contains the monoterpene aldehydes, and in addition, the alcohols linalol and terpinen-4-ol. This is because these compounds are eluted after neral and geranial and before citronellal, so that the fraction must contain these alcohols so as not to lose the citronellal.

As a result, the aldehydes are all clearly identifiable without any interference. Information on components present in trace amounts (e.g., hexanal, heptanal, hexadecanal and heptadecanal) can be obtained by HPLC–HRGC. The exact determination of these compounds can be a valuable way of testing the quality and genuineness of these oils. It is these components that contribute to the characteristic aroma of essential oils, which is impossible to imitate in synthetic formulations.

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