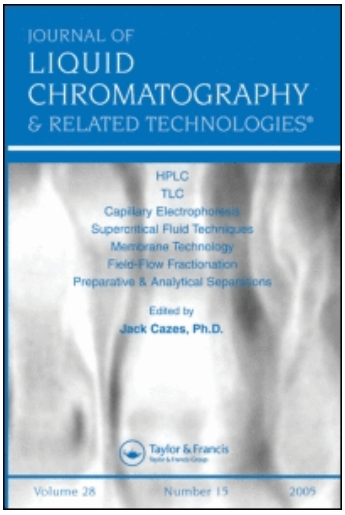

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 07 October 2002

To cite this Article Ogawa, C. A. , Diagone, C. A. and Lanças, F. M.(2002) 'SEPARATION OF MONOTERPENES IN ORANGE ESSENTIAL OIL BY CAPILLARY LIQUID CHROMATOGRAPHY AND MICELLAR ELECTROKINETIC CHROMATOGRAPHY', Journal of Liquid Chromatography & Related Technologies, 25: 10, 1651 — 1659
To link to this Article: DOI: 10.1081/JLC-120005712
URL: http://dx.doi.org/10.1081/JLC-120005712

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J. LIQ. CHROM. & REL. TECHNOL., 25(10&11), 1651–1659 (2002)

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ABSTRACT

This paper describes the evaluation of alternative techniques, for the determination of selected volatile compounds in cold-pressed orange oil. Two techniques were investigated: capillary-LC (C-LC) and micellar electrokinetic chromatography (MEKC). The separation by C-LC was performed on a 23 cm × 0.53 mm i.d. fused-silica column packed in house with RP-18. Acetonitrile : water (80 : 20) was used as mobile phase. The compounds were detected at 195 and 240 nm in a UV-detector with either a "Z" or "U" shaped flow cell.

In MEKC, 50 mM SDS was used in a 20 mM phosphate buffer at pH 8.1, and separations were carried out at 20 kV. A fused-silica capillary (75 μm i.d., 52.5 cm effective length, 60 cm

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total length) was used. On-column UV detection was conducted at 214 and 254 nm. A clean-up with C-18 as adsorbent and acetonitrile as eluent was performed before the chromatographic and electrophoretic runs in order to remove the colored components that could promote interference in the analysis.

Capillary-LC allowed the determination of terpenes such as mircene, limonene, α-pinene, and oxygenated terpenes including linalool, citral, and carvone while, in MEKC, carvone, mircene, linalool, limonene, and citral were determined.

Key Words: Orange essential oils; Monoterpenes; Capillary LC; MEKC

INTRODUCTION

Essential oils are complex mixtures of fragrance and flavor substances originating in plants. Many of these natural complexes are used as perfume or flavor components. The majority of the essential oils of commercial interest are mixtures of mono- and sesquiterpenoids, containing only minor amounts of compounds belonging to other classes.[1] The oils are commonly produced by cold pressing and contain more than 95% of monoterpene hydrocarbons, mainly limonene.[2] A number of monoterpenes have been shown to possess antimicrobial activities, and pharmaceutical preparations that exploit these properties are numerous. Furthermore, many therapeutic properties have been claimed for both essential oils and their isolated components; for example effects such as anti-inflammatory, carminative, hyperemic, sedative, expectorant, and the all-encompassing anti-helminthic activity have all been reported.[3] Although, high resolution gas chromatography (HRGC) has been the selected technique for the analysis of essential oils,[4–7] in this work, we have investigated the use of two alternative techniques to separate monoterpenes from orange essential oil: capillary-LC and MEKC.

EXPERIMENTAL

Apparatus

MEKC was performed at room temperature using a Waters Quanta 4000 CE System (Massachusetts, USA) equipped with an on-column UV detector. The chromatographic system used a Carlo Erba (Milan, Italy) micro-LC instrument designed for isocratic elution operations, with a packed capillary column. A Phoenix 20 syringe pump (Carlo Erba) with a capacity of 20 mL was used for the

eluent delivery. The samples were injected through a 60 nL internal-loop valve (Valco Instruments Texas, USA). A programmable variable wavelength UV-Vis detector, MicroUVIS 20 from Carlo Erba (Milan, Italy), fitted with either a "Z" shaped flow cell from Fisons (Milan, Italy), having 1.2 μL illuminated volume and 8 mm path length, or a "U" shaped flow cell from LC Packing (San Francisco, USA), having 35 nL illuminated volume and 8 mm path length, were employed. Data were collected and analyzed with a Chrom Card data acquisition system from Carlo Erba (Milan, Italy). The capillary columns were slurry packed in house, using a fused silica tubing (Siemens, Germany). The packing material employed was C18 from Alltech (Illinois, USA), with 5 μm particle diameter and 8 nm mean pore diameter not endcapped. GC-MS analysis was performed in a HP 5890 gas chromatograph, coupled to a HP 5970 MSD, both from Hewlett Packard (California, USA). The LM-5 (5% phenyl methyl polysiloxane) 50 m × 0.25 mm × 0.35 μm column was donated by LM Scientific (Sao Paulo, Brazil).

Chemicals

Monoterpenes (citral, linalool, α-terpineol, α-pinene, β-pinene, mircene, and carvone) were supplied by local citrus industries, and dissolved in either methanol or acetonitrile (see Fig. 1 for the structure of the selected terpenes). Methanol and acetonitrile were from E. M. Merck (Rio de Janeiro, Brazil). Monobasic potassium phosphate and sodium hydroxide were obtained from

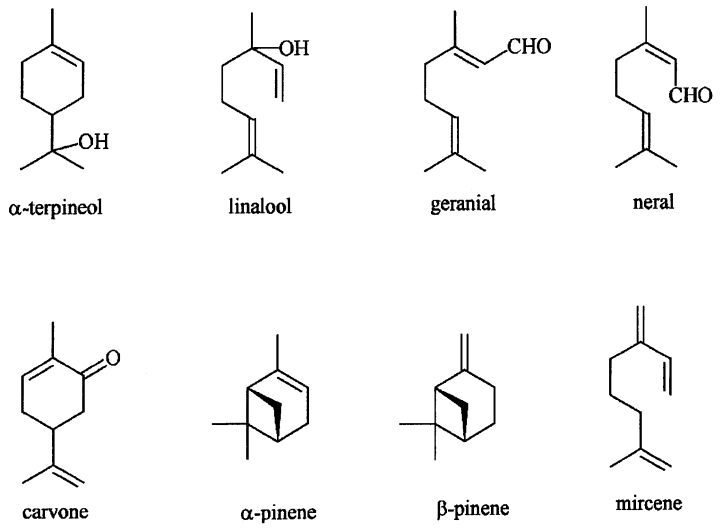

Figure 1. Structures of some terpenes present in essential oils.

Reagen (Rio de Janeiro, Brazil). Sodium dodecyl sulfate (SDS) was purchased from Polyscience (Illinois, USA). Water was purified in a Waters Millipore Milli-Q System (Eschborn, Germany). Cold-pressed orange oil was obtained from the Brazilian local citrus industry. An SPE cartridge containing 1 g of C-18 (lot 070840) from Varian (California, USA) was used for clean-up of the orange oil.

Procedures

The clean-up of 1 g crude essential orange oil was performed in a C-18 cartridge using 10 mL of the acetonitrile as eluent. Separations in MEKC were performed in 75 μm i.d. fused-silica capillary of 52.5 cm effective length and 60 cm total length, using a 20 mM phosphate buffer (pH 8.1) and 50 mM SDS as surfactant. The capillaries were washed with water for 5 minutes, then rinsed with 0.5 N NaOH and buffer for 10 minutes each. The experiments were carried out under an applied voltage of 20 kV, with samples being introduced into the column using a hydrodynamic mode (between 3 and 10 s).

The capillary-LC separations were performed in a (C-18 packed) fused-silica capillary, using acetonitrile–water (80/20) as the mobile phase at a flow rate of 5 μL/min. Detection was performed by monitoring the UV absorbance at 195 nm for the terpene hydrocarbons (mircene, α-pinene, β-pinene), alcohols (α-terpineol, linalool), and 240 nm for citral and carvone.

RESULTS AND DISCUSSION

Capillary-LC

In the present work, the major constituents of orange essential oil were analyzed and compared using two different techniques (MEKC and capillary-LC). Both Tables 1 and 2 list the terpenes identified in orange essential oil, using both capillary-LC and MEKC. Figures 2 and 3 show the chromatograms obtained by capillary-LC of the monoterpenes detected at 195 nm and 240 nm. Peaks were identified by matching their retention time with those of analytical standards, using information of the sample composition as obtained by GC/MS.

Many essential oils contain unsaturated hydrocarbons as major constituents; more than 90% peel oil contains D-limonene.[5,8] This compound is used as a fragrance material in perfuming household products and as a component of artificial essential oils.[1] Through capillary-LC, it was possible to identify this component, considering its high concentration as shown in the chromatogram (Fig. 2), typical retention time when compared with analytical standards, and its UV absorption at low wavelengths. The presence of limonene was also confirmed

Table 1. Identification of the Compounds Found in Essential Orange Oil by Capillary-LC and Detected at Different Wavelengths (see Figs 2 and 3)

Compound	195 nm	240 nm
1	Linalool	–
2	Citral	Citral
3	Mircene	Mircene
4	Limonene	Limonene
5	α-Pinene	–
6	–	Carvone

by the GC-MS data on the same sample. Mircene, considered the second major terpene in cold-pressed Valencia oil,[9] was identified as the second largest peak by capillary-LC. The pinenes are the most important, naturally occurring in varying ratios in essential oils. They are used in organic synthesis, as a fragrance substance to improve the odor of technical products. In this work, under the conditions used, only α-pinene was confirmed, since limonene overlapped with β-pinene, because the high concentration of the former, making difficult its identification.

Although terpene alcohols occur widely in nature, few of them have the physiological properties, which make them to be fragrance or flavor compounds. Several monoterpenes, including the alcohols linalool and α-terpineol, are important mediators in the chemical communication between organisms, and are essential in insect-plant interactions.[3,10] Off-flavors may be developed as a result of chemical microbiological or biochemical activity in fruits and juices.[7] α-Terpineol, that contributes to give off-flavor in citrus[7] and linalool, were not separated using the RP-18 capillary column under the investigated condition.

Table 2. Identification of the Compounds Found in Essential Orange Oil by MEKC and Detected at Different Wavelengths (see Figs 4 and 5)

Compound	214 nm	254 nm
1	Linalool	–
2	–	Citral (Geranial and Neral)
3	Mircene	Mircene
4	Limonene	Limonene
6	Carvone	Carvone

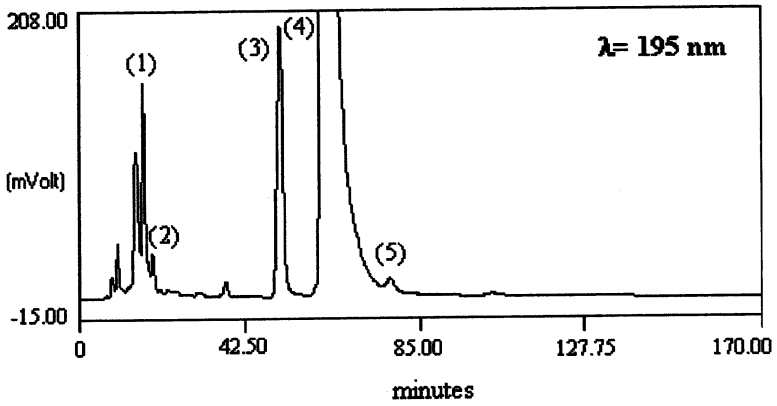

Figure 2. Orange oil analysis by capillary-LC. Column: RP-18 (23 cm × 0.53 mm × 5 μm), ACN/H_2O (80/20), flow rate: 5 μL/min. (1) linalool, (2) citral, (3) mircene, (4) limonene, (5) α-pinene. See Table 1.

These two compounds eluted together as a single peak. However, GC-MS analysis of the same sample showed only the presence of linalool. The terpenoid aldehydes and ketones are encountered as flavor constituents in practically all essential oils.[8] Citral and carvone were identified in the orange oil studied. Although carvone has not been directly associated with orange flavor, it can be used in synthetic citrus flavor,[5] while citral is one of the most important

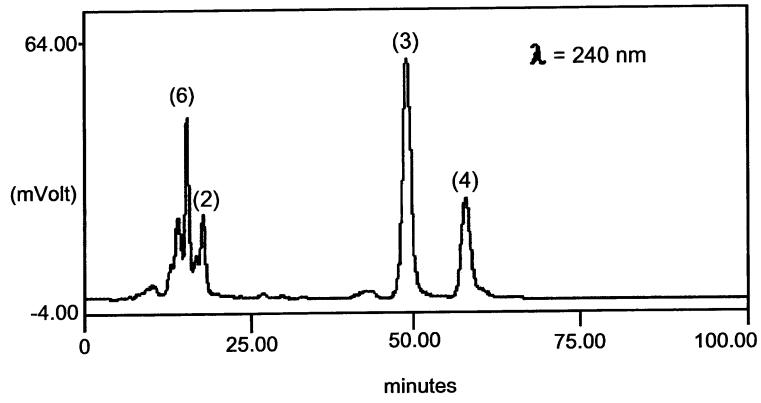

Figure 3. Orange oil analysis by capillary-LC. Column: RP-18 (23 cm × 0.53 mm × 5 μm), ACN/H_2O (80/20), flow rate: 5 μL/min. (2) citral, (3) mircene, (4) limonene, (6) carvone.

Table 3. Detection Limit (mg/L) for Monoterpenes Employing "Z" and "U" Shaped Flow Cells

Cell	Linalool	α-Terpineol	α-Pinene	β-Pinene	Carvone
"U" shaped	25	100	80	200	1
"Z" shaped	3	6	5	10	0.03

components expressing the typical lemon-like odor.[11] Capillary-LC proved to be a technique with good sensitivity for the analysis of these compounds. When the "Z" shaped flow cell was employed, a better detection limit relative to the "U" shaped flow cell (see Table 3) was obtained. Despite "Z" shaped flow cell having promoted peak dispersion around 7.5% relative to the "U" shaped flow cell, we can observe in Table 3 that the "Z" shaped flow cell showed the best detection. This was already expected since the illuminated volume was larger in the "Z" than "U" shaped flow cell.

Micellar Electrokinetic Chromatography (MEKC)

Six compounds that were separated in the orange essential oil by MEKC were detected at both wavelengths, 214 and 254 nm. The largest peak[4] shown in the electropherograms (Figs 4 and 5) was attributed to the limonene. The presence of limonene was also confirmed by GC-MS analysis of the same

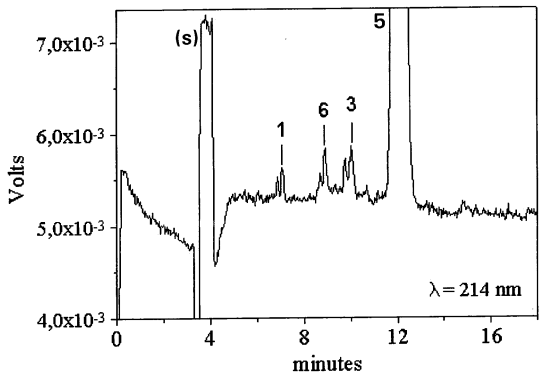

Figure 4. Electropherogram of orange oil obtained by MEKC at 214 nm. Potassium phosphate buffer 20 mM; pH 8.1; 50 mM SDS. (1) linalool, (3) mircene, (4) limonene, (6) carvone, (s) solvent. See Table 2.

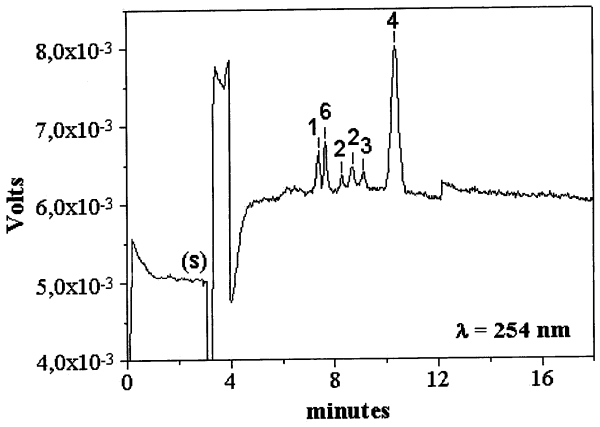

Figure 5. Electropherogram of orange oil obtained by MEKC at 254 nm. Potassium phosphate buffer 20 mM; pH 8.1; 50 mM SDS. (1) linalool, (2) citral (geranial or neral), (3) mircene, (4) limonene, (6) carvone, (s) solvent. See Table 2.

Table 4. Detection Limit (mg/L) for Monoterpenes Using MEKC

Citral	Linalool	α-Terpineol	α-Pinene	β-Pinene	Carvone
5	100	200	50	50	1

sample. The other components were identified by co-injection of analytical standards and confirmed by GC-MS analysis. These results are shown in Table 2. We could not specify, which isomers of citral (geranial or neral) were present since neither geranial or neral analytical standards were available separately in our laboratory.

Table 4 shows the detection limit obtained by MEKC. This technique presented a higher detection limit for most compounds relative to capillary-LC. However, MEKC presented a shorter analysis time (less than 15 min). Both techniques investigated in this work, capillary-LC and MEKC, show both eluent and sample saving. This pioneering work reveals that C-LC and MEKC are potential techniques for the analysis of citrus essential oils, and that further work aiming toward their optimization in this field should be stimulated.

ACKNOWLEDGMENTS

We thank CNP_q and FAPESP for financial support to our laboratory.

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Received January 12, 2002
Accepted February 12, 2002
Manuscript 5758