

Characterization of Essential Oils from Lamiaceae Species by Fourier Transform Raman Spectroscopy

DIMITRA J. DAFERERA, PETROS A. TARANTILIS, AND MOSCHOS G. POLISSIOU*

Laboratory of General Chemistry, Agricultural University of Athens, 75 Iera Odos,
 118 55 Athens, Greece

The Fourier transform Raman (FT-Raman) spectra of pure terpenes and essential oils obtained by hydrodistillation of some Lamiaceae species, are presented. This study shows that principal components of an essential oil can be recognized by FT-Raman. Components predicted by FT-Raman spectrum of an essential oil correlate well with those found as major constituents by GC-MS. In this way the basic chemical character of an essential oil can be recognized. The results demonstrate that certain Raman intensities can be correlated to specific terpenes and therefore FT-Raman can discriminate between the essential oils of which main components belong to different classes of compounds.

KEYWORDS: FT-Raman; GC-MS; Lamiaceae; essential oils

INTRODUCTION

Essential oils are natural mixtures of terpenes, most of which are obtained from aromatic and pharmaceutical plants. The chemical composition of the essential oils differs for each species or subspecies and is characteristic for each. For example, linalool and its corresponding ester characterize the lavender essential oil, while carvacrol is the predominant compound in dictamnus. Furthermore, among the same subspecies, there are different chemotypes due to environmental, seasonal, or other factors, which are responsible for compositional changes in the essential oils (1–4). Thus, there are two known chemotypes for marjoram essential oil, the *cis*-sabinene hydrate/terpinen-4-ol and the carvacrol/thymol types (5–6).

Gas chromatography-mass spectrometry (GC-MS) is the most popular method of studying essential oil composition, which allows the identification of the specific terpenes found in an essential oil by comparing their relative retention times and their mass spectra. Fourier transform Raman (FT-Raman) spectroscopy is an analytical technique based on the interaction of an incident monochromatic radiation with vibrational energy levels of molecules. It is used for qualitative comparisons between samples because it is a rapid, simple, and nondestructive method for the sample (7).

Identification of individual components of complex mixtures, such as terpenes in essential oils, requires the use of several techniques. Infrared spectroscopy is used in combination with GC and/or GC-MS analysis for the identification of essential oil components (8, 9). Recently, near-infrared and Raman vibrational spectroscopies have been effectively applied to the identification of such compounds in essential oils without any previous separation (10, 11).

In the present work, the FT-Raman spectra of a number of pure terpenes and their natural mixtures in essential oils from different plant species of the Lamiaceae family have been studied in an effort to correlate them with their chemical composition. In addition, the possibility of the essential oil discrimination between different plant species, subspecies, or chemotypes by their FT-Raman spectra has been examined.

MATERIALS AND METHODS

Materials. Pure α -pinene, β -myrcene, cineole, α -terpinene, γ -terpinene, *p*-cymene, limonene, terpineol, linalool, *d*-camphor, borneol, thujone, menthone, pulegone, thymol, carvacrol, and *trans*-caryophyllene were purchased from the Sigma-Aldrich Co (St. Louis, Missouri). Naturally growing plants of oregano (*Origanum vulgare*), thyme (*Thymus capitatus*), dictamnus (*Origanum dictamnus*), marjoram (*Origanum majorana*), sage (*Salvia fruticosa*), and pennyroyal (*Mentha pulegium*) were collected from specific areas in Greece, air-dried, and stored at room temperature (20–25 °C) in darkness until distillation. Lavender (*Lavandula angustifolia*) was purchased from a local market in Athens.

Isolation of the Essential Oils. The classic method of hydrodistillation using the Clevenger apparatus for 4 h was used for the isolation of the essential oils from oregano, thyme, marjoram, sage and pennyroyal leaves and flowers, dictamnus leaves, and lavender flowers. The isolated essential oils in pure form were stored at –18 °C until their analysis by GC-MS.

GC-MS Analysis Conditions. The analysis of the essential oils was performed using a Hewlett-Packard 5890 II GC, equipped with a 30 m \times 0.25 mm id, 0.25 μ m HP-5MS capillary column, and a HP 5972 mass selective detector. Helium was the carrier gas at 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. Column temperature was set at 40 °C for 3 min, then programmed from 40 to 180 °C at a rate of 3 °C/min, and finally increased to 270 °C at 30 °C/min and held for 5 min. For GC-MS detection an electron ionization system was used with ionization energy of 70 eV. Diluted samples of 0.5 μ L (1/100 in acetone) were injected manually and splitless.

* Corresponding author. Telephone: ++ 30 1 0529 42 41. Fax: ++ 30 1 0529 42 65. E-mail: mopol@aua.gr.

Table 1. Main Components of the Essential Oils of Oregano (O1, O2), Thyme (T), Dictamnus (D1, D2) and Marjoram (M1, M2)

component	% composition							
	O1	O2	T	D1	D2	M1	M2	
thujene ^a	0.5	0.4	0.5	0.2	0.2	0.9	3.3	
α -pinene ^b	0.4	0.5	0.8	0.4	0.5	0.7	2.5	
camphene ^a	0.1	—	0.1	—	—	tr ^c	1.3	
sabinene ^b	—	—	—	—	—	0.4	1.5	
β -myrcene ^b	0.9	1.0	1.1	0.5	0.5	1.3	3.6	
α -terpinene ^b	0.3	1.4	0.8	1.1	1.1	5.4	9.8	
<i>p</i> -cymene ^b	5.2	13.0	5.4	15.1	20.3	5.8	9.8	
β -phellandrene ^a	0.3	—	tr ^c	0.3	—	1.8	3.8	
γ -terpinene ^b	5.8	7.5	2.6	5.4	3.9	9.3	18.3	
<i>cis</i> -sabinene hydrate ^a	—	—	—	—	—	1.3	1.4	
terpinolene ^a	tr ^c	0.1	0.2	—	—	1.5	2.0	
linalool ^b	0.2	—	0.4	0.6	1.3	5.6	2.3	
borneol ^b	—	—	0.2	0.1	0.1	0.5	1.6	
terpinen-4-ol ^a	—	0.3	0.5	0.6	0.9	9.4	9.3	
α -terpineol ^b	tr ^c	—	tr ^c	—	—	2.4	2.6	
linalyl propanoate ^a	—	—	—	—	—	1.4	—	
linalyl acetate ^a	—	—	—	—	—	0.8	1.2	
thymol ^b	1.8	63.7	0.2	0.3	2.6	0.8	0.1	
carvacrol ^b	80.8	8.6	81.5	72.1	64.1	45.1	16.8	
caryophyllene ^b	1.1	1.0	2.7	0.9	1.0	1.0	0.3	
total	97.4	97.5	96.5	97.6	96.5	97.2	91.5	

^a Tentatively identified by the library of the GC-MS system. ^b Identified by standard. ^c tr, <0.09%.

Identification of the components was based on their relative retention time and their mass spectra in comparison with those observed by standards. Some compounds were tentatively identified by using the NBS75K library data of the GC-MS system. The elution order of the compounds is in agreement with their relative retention indices on nonpolar phases reported in the literature (12).

FT-Raman Spectroscopy. FT-Raman spectra of pure compounds and essential oils were recorded with a Nicolet 750 FT-Raman spectrometer, equipped with a Nd:YAG laser source that emits at 1064 nm. A calcium fluoride (CaF₂) beam splitter, an indium–gallium arsenide (InGaAs) detector, and 180° backscattering geometry are used in the spectrometer. An optical bench alignment was performed before each batch of measurements to ensure that the spectrometer was fine-tuned and the detector signal maximized. Sample cells used were cut to 6 cm from Wimad WG-5M NMR tubes of 4.97 mm outer diameter and 0.38 mm wall thickness. A motorized positioner focused the laser beam to the sample, and a manual side-to-side adjuster allowed sample adjustment for maximum optical efficiency. Spectra were accumulated from 100 scans collected during 3 min at a resolution of 4 cm using the built-in software of the spectrophotometer (OMNIC 3.1).

RESULTS AND DISCUSSION

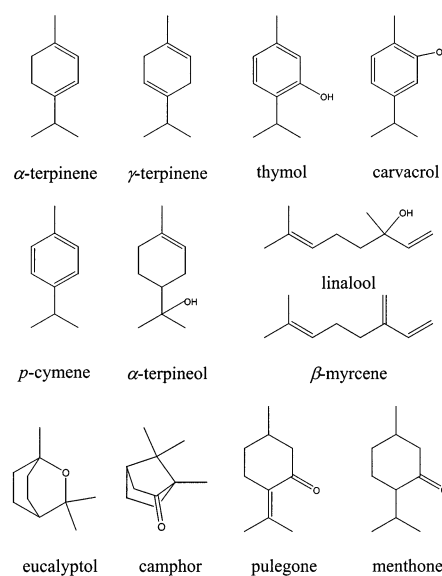
The chemical composition of the essential oils was determined by the GC-MS analysis. In the cases of oregano, dictamnus, and marjoram oils, two different plant materials of each species were examined. The results of oregano, thyme, dictamnus, and marjoram essential oils are presented in **Table 1**. The GC-MS analysis of the essential oils from the two plant materials of oregano showed that they belong to different chemotypes, carvacrol (O1) and thymol (O2). The results between the two plant materials of dictamnus (D1, D2) were similar. In the case of the two marjoram samples, the qualitative composition was the same, but quantitative differences were observed (**Table 1**). The results of lavender, sage, and pennyroyal essential oils are presented in **Table 2**. Compounds that participated less than 0.1% in the mixtures did not present in both tables.

The molecular structures of some characteristic terpenes are presented in **Figure 1**. The recorded FT-Raman spectra of standard compounds and essential oils are presented in **Figures**

Table 2. Main Components of the Essential Oils of Lavender (L), Sage (S), and Pennyroyal (P)

component	% composition		
	L	S	P
α -pinene ^a	—	6.3	0.8
camphene ^b	0.5	1.7	—
β -pinene ^b	—	2.4	0.9
3-octanone ^b	0.1	—	—
β -myrcene ^a	1.6	2.6	—
<i>p</i> -cymene ^a	0.5	1.3	—
limonene ^a	—	—	0.8
eucalyptol ^a	2.4	59.5	0.2
(<i>z</i>) β -ocimene ^b	0.7	—	—
α -ocimene ^b	1.1	—	—
γ -terpinene ^a	0.1	—	—
linalool oxide A (furanoid) ^b	3.0	—	—
linalool oxide B (furanoid) ^b	2.1	—	—
linalool ^a	25.5	1.5	—
thujone ^b	—	3.1	—
3,7-dimethyl-1,5,7-octatrien-3-ol ^b	2.1	—	—
octen-1-ol, acetate ^b	1.6	—	—
1,3,8- <i>p</i> -menthatriene ^b	0.6	—	—
camphor ^a	0.6	10.2	—
<i>trans</i> -menthone ^a	—	—	1.2
<i>cis</i> -menthone ^b	—	—	15.0
<i>trans</i> -pinocamphone ^b	—	0.7	—
borneol ^b	3.2	0.2	—
terpinen-4-ol ^b	1.7	0.6	—
cryptone ^b	1.4	—	—
α -terpineol ^a	5.6	3.9	—
1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde ^b	—	—	2.3
pulegone ^a	—	—	76.6
nerol ^a	1.0	—	—
linalyl acetate ^b	17.7	—	—
bornyl acetate ^b	1.0	0.1	—
lavandulyl acetate ^b	3.9	—	—
thymol ^a	3.2	1.2	0.4
carvacrol ^a	3.2	0.7	0.1
piperitenone ^b	—	—	0.6
neryl acetate ^b	1.3	—	—
geranyl acetate ^b	2.6	—	—
caryophyllene ^a	1.0	0.4	—
caryophyllene oxide ^b	3.3	—	—
total	92.6	96.4	98.9

^a Identified by standard. ^b Tentatively identified by the library of the GC-MS system.

**Figure 1.** Molecular Structures of some characteristic terpenes.

2–4. There are differences between the spectra of standards and between the spectra of essential oils, whose predominant components belong to different classes of compounds. The FT-

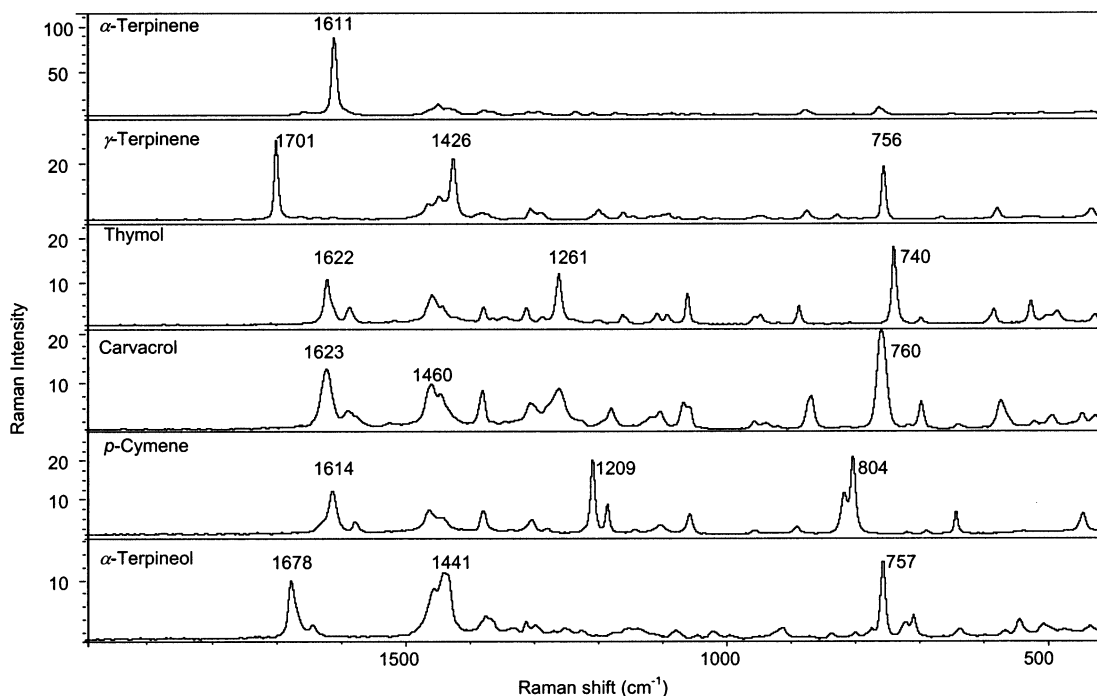


Figure 2. FT-Raman spectra of α -terpinene, γ -terpinene, thymol, carvacrol, *p*-cymene, and terpineol.

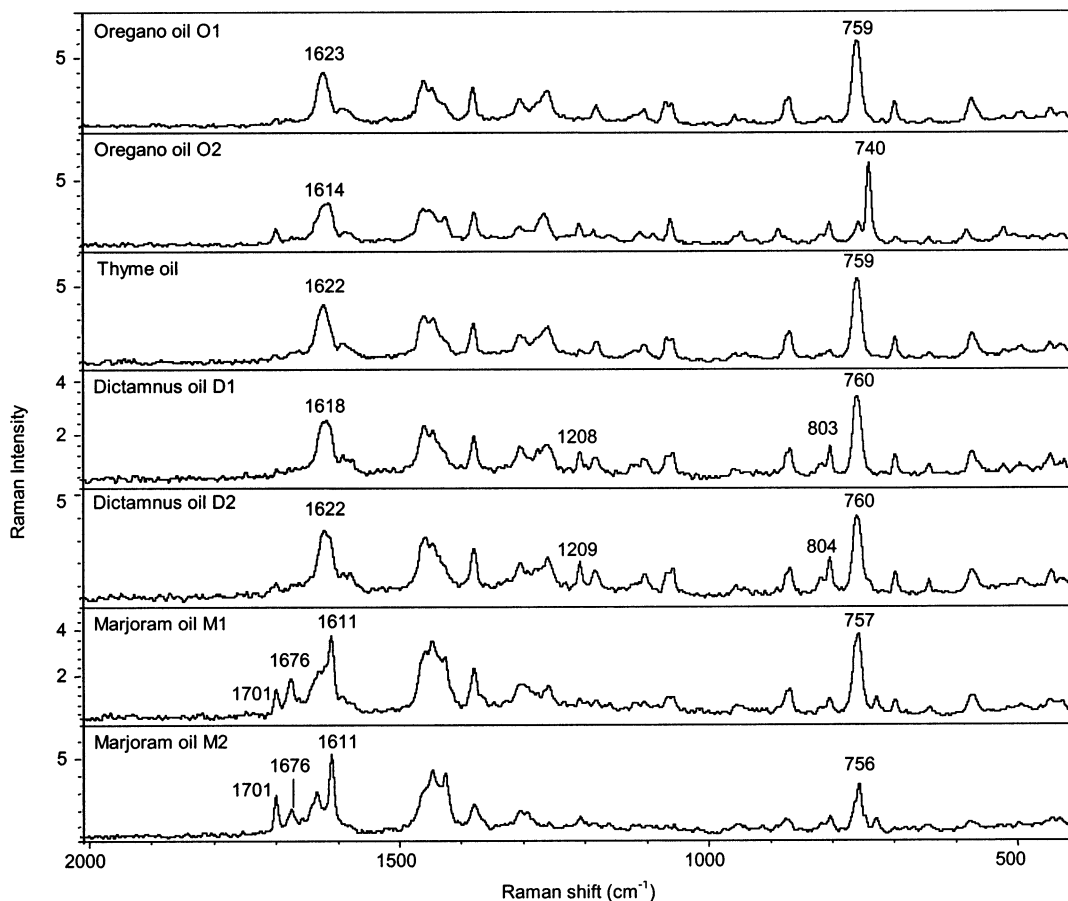


Figure 3. FT-Raman spectra of oregano O1 and O2, thyme, dictamnus D1 and D2, and marjoram M1 and M2 essential oils.

Raman spectrum of each terpene is characteristic of the molecule structure. As a result, the FT-Raman spectra of essential oils in which the same component predominated displayed similar profiles.

Important differences were obtained for the FT-Raman spectra of the isomeric compounds α -terpinene and γ -terpinene, and

thymol and carvacrol (Figure 2). The characteristic peak for α -terpinene appeared at 1611 cm^{-1} (conjugated C=C of cyclohexadiene) and for γ -terpinene at 1701 cm^{-1} (nonconjugated C=C of cyclohexadiene). In the case of thymol, the FT-Raman spectrum showed a characteristic peak at 740 cm^{-1} (ring vibration), while the corresponding one for carvacrol appeared

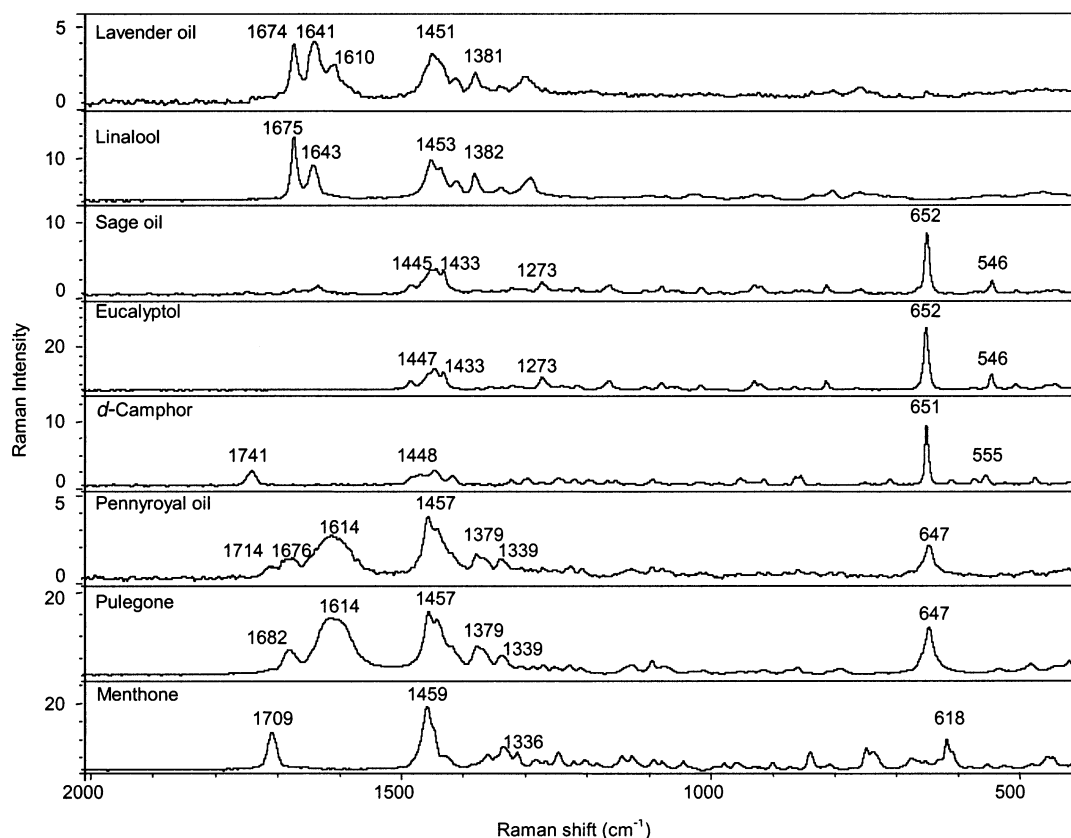


Figure 4. FT-Raman spectra of lavender essential oil, linalool, sage essential oil, eucalyptol, *d*-camphor, pennyroyal essential oil, pulegone, and menthone.

at 759 cm^{-1} . The above compounds, in combination with *p*-cymene, constituted the majority of the essential oils of oregano, thyme, dictamnus, and marjoram (Table 1).

FT-Raman spectra of oregano O1, thyme, and dictamnus essential oils showed similar profiles to each other and to their main constituent carvacrol (Figure 3). The more intense peak at 804 cm^{-1} appeared in the dictamnus spectrum is attributed to *p*-cymene (ring breathing), which participated in dictamnus composition in higher percentages than the others (Table 1). Although carvacrol is one of the main components found in marjoram essential oil, the FT-Raman spectrum of marjoram is differentiated due to the presence of other compounds with intense Raman signals, such as linalool and β -myrcene. As a result, there are qualitative differences from 1700 to 1550 cm^{-1} and to the shape of the 756 cm^{-1} peak. The peaks at 1701 and 1611 cm^{-1} are attributed to the isomers γ -terpinene and α -terpinene, respectively, while that at 1676 cm^{-1} is due to linalool and β -myrcene ($\text{C}=\text{C}$ of $\text{RC}=\text{C}(\text{CH}_3)_2$). The shape of the band between 760 and 756 cm^{-1} indicated the presence more than one substance. The FT-Raman spectra of γ -terpinene and α -terpineol showed the characteristic peak about 757 cm^{-1} , and in combination with carvacrol, they contributed to its appearance in the marjoram spectrum. Notable qualitative differences were observed between the FT-Raman spectra of two oregano chemotypes. The spectrum of the carvacrol chemotype displayed the more intense and characteristic peak at 759 cm^{-1} , while in the thymol chemotype, the same peak shifted to 740 cm^{-1} (Figure 3).

FT-Raman spectra of lavender, sage, and pennyroyal essential oils were simpler, compared with the spectra of oregano, thyme, dictamnus, and marjoram essential oils. FT-Raman spectra of lavender essential oil showed characteristic peaks at 1674 and 1641 cm^{-1} , due to the presence of linalool ($\text{C}=\text{C}$ of $\text{RC}=\text{C}(\text{CH}_3)_2$ and $\text{RC}=\text{CH}_2$, respectively) in its mixture (Figure 4).

The peak at 1610 cm^{-1} may be attributed to the ester of linalool ($\text{C}=\text{C}$ of $\text{R}-\text{O}-\text{C}(=\text{O})-\text{C}=\text{CH}_2$). The FT-Raman spectra of sage and pennyroyal essential oils are representative of their predominant compounds. The FT-Raman spectrum of sage oil was characterized by a triple peaks between 1500 and 1400 cm^{-1} and a very strong peak at 652 cm^{-1} , which is representative for the bicyclocamphane-type compounds, eucalyptol and camphor (Figure 4). In the FT-Raman spectrum of pennyroyal essential oil, the peak at 1714 cm^{-1} is due to menthone, and the peaks at 1614 and 647 cm^{-1} are attributed to pulegone, while the peak at 1457 cm^{-1} belongs to both to them (Figure 4).

The FT-Raman spectrum of an essential oil represents a fingerprint for it, exhibiting the characteristic profile of its main constituents. Compounds which participated in an essential oil composition in relatively low percentages did not seem to influence the FT-Raman spectrum. Therefore, it is possible to discriminate between essential oils whose main components belong to different classes of compounds. The results of FT-Raman spectra are in agreement with the GC-MS data, and they contributed to the identification of the compounds, particularly in cases in which isomeric compounds have the same mass spectra and eluted at very close times, as with thymol and carvacrol. FT-Raman spectroscopy can therefore be applied for control of essential oil quality as a very rapid and nondestructive method.

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