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Quantitative analysis of α-pinene and β-myrcene in mastic gum oil using FT-Raman spectroscopy

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Abstract

α-Pinene and β-myrcene are compounds that are contained in mastic gum in high concentrations. The β-myrcene percentage determines the marketability of mastic gums. The chemical composition of mastic gum oil of a representative resin quality was evaluated by gas chromatography–mass spectrometry (GC–MS) technique. FT-Raman spectroscopy, based on band intensity measurements, was used for the determination of α-pinene and β-myrcene content in mastic gum. Bands at 1658 and 1633 cm^{-1} were used for the calibration of α-pinene and β-myrcene, respectively. Calibration curves were linear (correlation coefficient for α-pinene was 0.992 and 0.997 for β-myrcene) in the range 30–80 and 3–45%, respectively. Normalization of calibration curves, against the 802 cm^{-1} cyclohexane band, minimized the effect of laser beam power fluctuations. The proposed method is rapid and simple. Accordingly, mastic gum oils from Chios island (Greece) contained 38.1–69.5% α-pinene and 4.5–57.9% β-myrcene. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: α-Pinene, β-Myrcene, Determination, FT-Raman

1. Introduction

Pistacia lentiscus var. *Chia*, is a tree belonging to the Anacardiaceae family, which is traditionally cultivated in south Chios, a Greek island of the east Aegean sea. Every year, from July to October, on the trunk of the tree, cuttings are made and a resinous liquid substance is exuded. This material, remaining under the tree for many days, is coagulated by the local environmental conditions. The coagulated product is collected and is called gum mastic or "masticha" (Perikos, 1993). Masticha has numerous usages itself, but is also used in producing gum oil by the steam-distillation method. Gum mastic oil is used in cosmetics and perfumery, as a flavouring in food technology and for its antimicrobial activity, and especially against *Helicobacter pylori* (Huwez, Thirwell, Cockayne, & Ala'Alden, 1998; Magiatis, Melliou, Skaltsounis, Chinou, & Mitaku, 1999; Perikos, 1993).

The gum essential oil chemical composition varies and depends on the gum quality. The gum quality is influenced by its purity, the collection time and the duration between exudation from the trunk and the collection. Studies on the chemical composition of the gum oil showed the predominant presence of monoterpenes, α-pinene and β-myrcene, which constitute the majority of the oil. The α-pinene and β-myrcene contents in mastic gum oil are typically measured by gas chromatography (GC) (Papageorgiou, Mellidis, & Argyriadou, 1991). Time-consumption and the prior sample handling are the basic disadvantages of the method. There is a proportion between the concentrations of α-pinene and β-myrcene, which characterizes the authenticity of the gum essential oil. Percentages of about 60–80% for α-pinene and 7–20% for β-myrcene represent an acceptable oil quality. Increases of β-myrcene in the oil mixture devalue its quality.

FT-Raman spectroscopy is an analytical technique based on the interaction of an incident monochromatic radiation with vibrational energy levels of molecules. It is widely used for qualitative comparisons between samples. The concentration of a compound in a mixture is relatable to the Raman intensities if an appropriate

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reference material is used to determine its value (Freeman & Mayo, 1969; Hancewicz & Petty, 1995; Skoulika, Georgiou, & Polissiou, 2000; Sun, Ibrahim, Oldham, Schultz, & Conners, 1997). FT-Raman spectra of α-pinene have been recorded in order to determined the quantitative relationship between vibrational circular dichroism (VCD) and Raman optical activity (ROA) spectra (Qu, Lee, Yu, Freedman, & Nafie, 1996).

This paper describes the development of a method for quantitative analysis of α-pinene and β-myrcene in mastic gum oil by FT-Raman spectroscopy. The recorded FT-Raman spectra of the gum mastic oils allow us to correlate their chemical compositions with the percentages of α-pinene and β-myrcene. The proposed method is simple, rapid and nondestructive for the sample and all samples are measured as neat liquids without further treatment.

2. Materials and methods

2.1. Materials

A sample of first quality mastic gum and 10 mastic gum oils from resins collected over different durations were kindly provided by the Chios gum mastic growers' association. The 10 samples were randomly characterized as "oil 1", "oil 2", "oil 3", "oil 4", "oil 5", "oil 6", "oil 7", "oil 8", "oil 9" and oil 10". Standards of α-pinene and β-myrcene were purchased from the Sigma-Aldrich Co.

2.2. Isolation of essential oil from the mastic gum

A typical essential oil was isolated according to the Likens–Nickerson's method, using a micro steam distillation extraction apparatus for organic solvents lighter than water (Daferera, Ziogas, & Polissiou, 2000). In that way, all the aroma constituents were concentrated in the extracting solvent. The procedure was protected by placing inert gas (N_2) in the main body of the apparatus in order to avoid creating oxidized by-products. The diethylether extract was stored at 4 °C until its analysis by gas chromatography–mass spectrometry (GC–MS).

2.3. GC–MS analysis conditions

The analysis of the typical gum essential oil was performed using a Hewlett Packard 5890 II GC, equipped with a HP-5 capillary column (30 m, 0.25 mm id, 0.25 μm film thickness) and a mass spectrometer 5971 A as detector. The carrier gas, helium, was stable at 2.5 psi. Column temperature was initially kept for 3 min at 40 °C, then gradually increased to 180 °C at 3 °C/min and finally increased to 270 °C at 30 °C/min. Injector and detector (MS transfer line) temperatures were set at 220 and 290 °C, respectively. For GC–MS detection, an electron ionization system was used with ionization energy of 70 eV. The extract diluted 1:5 (v/v) with diethylether and 1.0 μl of the diluted sample was injected automatically and split-less.

The samples from the Chios gum mastic growers' association were also analyzed by the above GC–MS method in order to determine the α-pinene and β-myrcene. The samples diluted with dichloromethane (1:100, v/v) and 1 μl of the diluted sample was injected automatically and split less.

2.4. FT-Raman spectroscopy

Standard solutions were prepared using the standards, α-pinene and β-myrcene, in dichloromethane.

FT-Raman spectra of cyclohexane, standard solutions, and ten mastic gum oils from the Chios gum mastic growers' association were recorded with a Nicolet 750 FT-Raman spectrometer, equipped with a Nd:YAG laser source that emits at 1064 nm. A calcium fluorine (CaF_2) 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 focuses the laser beam to the sample and a manual side-to-side adjuster allows sample adjustment for maximum optical efficiency. Spectra were accumulated from 100 scans collected during 3 min at a resolution of 4 cm^{-1}.

The FT-Raman spectra were smoothed and their baselines were corrected using the "automatic smooth" and the "automatic baseline correct" functions of the built-in software of the spectrophotometer (OMNIC 3.1). Then the intensities of the 1658 and 1633 cm^{-1} peaks were measured. The intensity of the 802 cm^{-1} cyclohexane peak was measured as well.

3. Results and discussion

3.1. GC–MS results

A typical total ion chromatogram (TIC) of the mastic gum oil, isolated by the Likens-Nickerson method, is presented in Fig. 1. This mastic gum oil is characterized by the presence of α-pinene (72.1%), β-pinene (2.9%), β-myrcene (16.5%), limonene (1.0%), linalool (1.0%) and caryophyllene (1.1%). All other compounds were present at relative concentrations less than 1.0% in the mixture (Table 1).

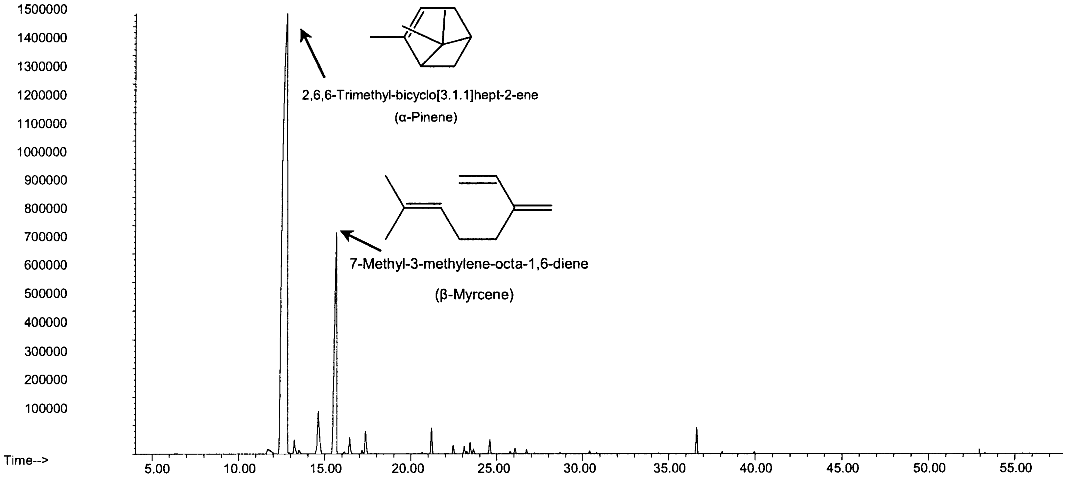

Fig. 1. A representative TIC of gum oil isolated with diethylether by the Likens–Nickerson method.

Table 1
Chemical composition of the essential oil from gum of *Pistacia lentiscus* var. *chia* isolated by the Likens–Nickerson method

A/A	Retention time (min)	Component	Composition (%)
1	11.70	4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane(sabinene or 4(10) thujene)	0.6
2	12.82	2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene(α-pinene)	72.1
3	13.22	2,2-dimethyl-3-methylene-bicyclo[2.2.1]heptane(camphene)	0.7
5	14.62	6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane(β-pinene)	2.9
6	15.67	7-methyl-3-methylene-1,6-octadiene (β-myrcene)	16.5
7	16.44	1-methoxy-2-methyl-benzene (o-cresol- methyl-ether)	0.7
8	17.16	1-methyl-4-(1-methylethyl)-benzene(p-cymene)	0.2
9	17.36	1-methyl-4-(1-methylethenyl)cyclohexene (limonene)	1.0
10	21.21	3,7-dimethyl-1,6-octadien-3-ol (linalool)	1.0
11	22.48	2,2,3-trimethyl-3-cyclopentene-1-acetaldehyde(α-campholene aldehyde)	0.3
12	23.10	6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptan-3-ol	0.3
13	23.27	4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-ol	<0.1
17	25.79	α,α,4-trimethyl-3-cyclohexene-1-methanol (a-terpineol)	0.1
18	26.07	6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol	0.2
19	26.74	4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-one(d-verbenone)	0.2
20	30.41	1-methoxy-4-(1-propenyl)-benzene (anethole)	0.1
21	36.63	4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene(caryophyllene)	1.1
22	38.11	2,6,6,9-tetramethyl-1,4,8-cycloundecatriene(a-caryophyllene or a-humulene)	0.1
23	39.96	1,2-dimethoxy-4-(1-propenyl)-benzene(methyl-isoeugenol)	<0.1
		Others not identified	1.7
		Total	100

Table 2 shows the percentages of α-pinene and β-myrcene in the samples from the Chios gum mastic growers' association, as determined by the GC–MS analysis. The contents (%) of α-pinene and β-myrcene in mastic gum oils were 33.7–72.8 and 3.8–63.5, respectively.

The chemical composition of the oils was determined by comparing the mass spectra of oil components with those of mass spectra from the NBS75K data library. The main components of the oil (α-pinene and β-myrcene) were also determined by comparison of their relative retention times with those of standards.

3.2. FT-Raman results

The FT-Raman spectrum (Fig. 2) of α-pinene showed characteristic peaks at 1658 cm^{-1} (C=C of cyclohexene), 1436 cm^{-1} (C–H) and 667 cm^{-1} (C–C ring breathing), β-myrcene at 1633 cm^{-1} ($R_1R_2C{=}CH_2$ and $R_1R_2C{=}CHR_3$) (Bour, 1998; Freeman & Mayo, 1969) and of cyclohexane at 802 cm^{-1} (C–C, cyclohexane chair form). The FT-Raman spectrum of mastic gum oil showed characteristic peaks at 1658 cm^{-1}, assigned mainly to α-pinene and at 1633 cm^{-1}, assigned to β-myrcene. It was observed that the Raman intensities of

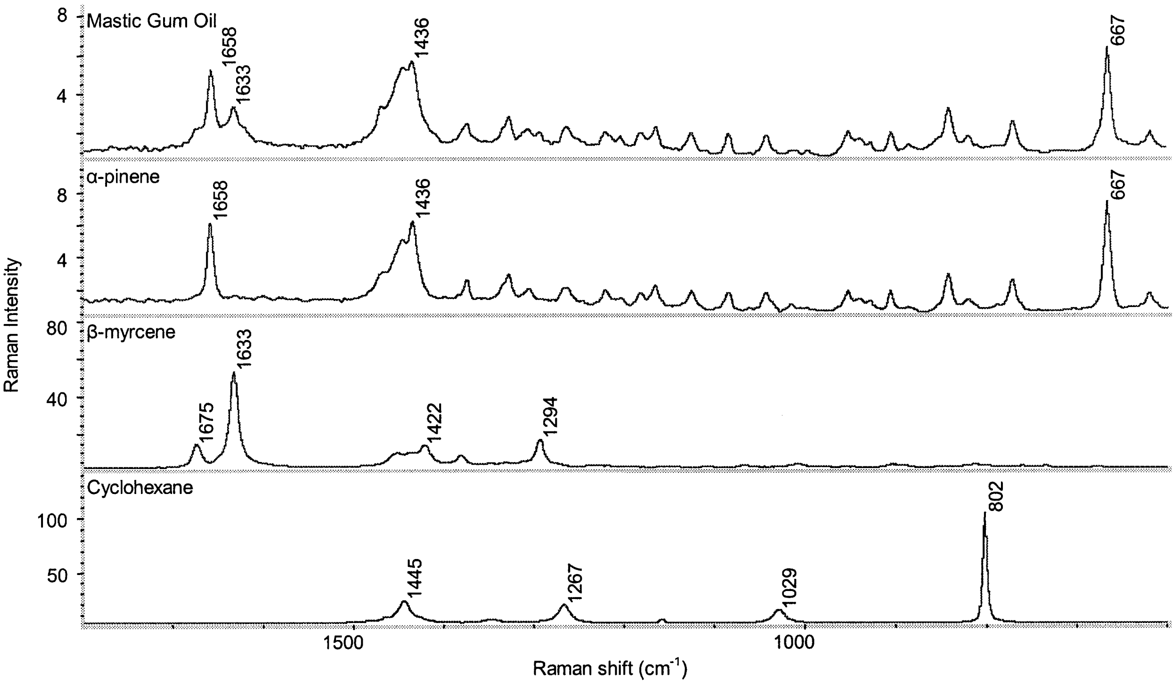

Fig. 2. FT-Raman spectra (1800–600 cm^{-1}) of mastic gum oil, α-pinene, β-myrcene and cyclohexane.

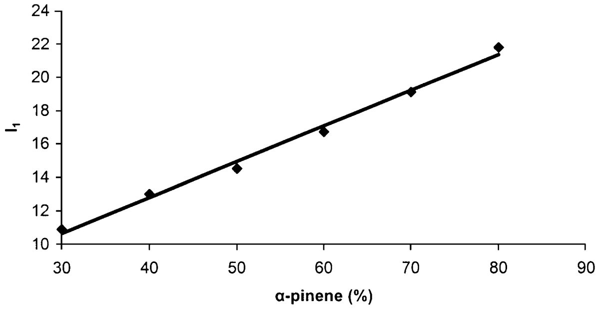

Fig. 3. The calibration curve of α-pinene.

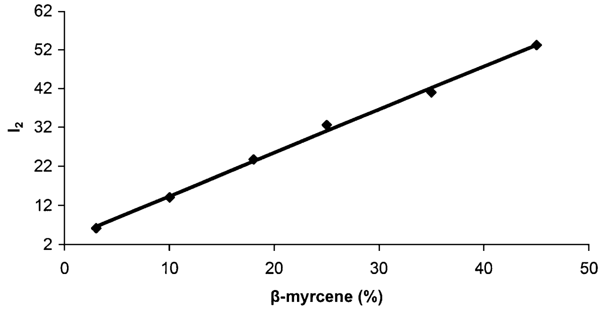

Fig. 4. The calibration curve of β-myrcene.

Table 2
The percentages of α-pinene and β-myrcene in samples from the Chios gum mastic growers' association, as determined by GC–MS analysis

Mastic gum oil	α-pinene (%)	β-myrcene (%)
Oil 1	70.4	22.9
Oil 2	72.8	6.0
Oil 3	65.6	26.9
Oil 4	59.1	33.5
Oil 5	63.2	28.6
Oil 6	54.0	3.8
Oil 7	68.4	25.4
Oil 8	64.9	28.3
Oil 9	71.5	19.4
Oil 10	33.7	63.5

Table 3
Contents (%) of α-pinene standards correlated with normalized intensity at 1658 cm^{-1}

Content (%) of α-pinene	(%) Normalized intensity at 1658 cm^{-1} ±S.D. ($n=3$)
30.0	10.9±0.4
40.0	13.0±0.8
50.0	14.5±0.2
60.0	16.7±0.6
70.0	19.1±0.4
80.0	21.8±0.9

the same sample fluctuated after closing–opening of the Raman source. Normalization minimizes the effect of laser power fluctuations (Skoulika et al., 2000). So the percent relative intensities of 1658 and 1633 cm^{-1} to the 802 cm^{-1} cyclohexane were measured for every standard solution and oil sample. Percent normalized intensities of 1658 cm^{-1} (I_1) and 1633 cm^{-1} (I_2) were correlated with α-pinene and β-myrcene contents, respectively. There were two linear relationships: one between I_1 and α-pinene content, and a second between I_2 and β-myrcene (Tables 3 and 4; Figs. 3 and 4). Empirical equations of calibration curves are,

Table 4
Contents (%) of β-myrcene standards correlated with normalized intensity at 1633 cm^{-1}

Content (%) of β-myrcene	(%) Normalized intensity at 1633 cm^{-1} ±S.D. ($n=3$)
3.0	6.2±0.1
10.0	14.1±0.4
18.0	23.8±0.7
25.0	32.6±0.5
35.0	41.0±0.7
45.0	53.3±0.7
65.0	81.2±0.3

Table 5
Contents (%) of α-pinene and β-myrcene of mastic gum oils measured by the FT-Raman spectroscopic method

Mastic gum oil	Normalized intensity at 1658 cm^{-1}	Normalized intensity at 1633 cm^{-1}	α-Pinene content (%)	β-Myrcene content (%)
Oil 1	17.5	23.8	63.3±1.4	18.5±0.2
Oil 2	18.8	11.3	69.5±1.9	7.2±0.8
Oil 3	17.8	34.3	64.8±1.1	27.9±0.7
Oil 4	17.7	39.0	64.3±3.4	32.2±1.0
Oil 5	16.4	32.5	58.1±1.2	26.3±0.5
Oil 6	14.5	8.3	49.0±0.8	4.5±0.1
Oil 7	18.1	31.7	66.2±1.0	25.6±0.6
Oil 8	17.8	33.5	64.8±1.6	27.2±0.5
Oil 9	16.4	22.4	58.1±1.7	17.2±0.2
Oil 10	12.2	67.6	38.1±1.2	57.9±1.7

for α-pinene:

$$I_1 = (4.2 \pm 0.6) + (0.21 \pm 0.01) \times \alpha\text{-pinene } (\%)$$

$$(r = 0.882, \quad n = 3)$$

for β-myrcene:

$$I_2 = (3.3 \pm 0.8) + (1.11 \pm 0.03) \times \beta\text{-myrcene } (\%)$$

$$(r = 0.997, \quad n = 3)$$

The % RSD fluctuated from 1.4 to 6.2% for the calibration curve of α-pinene and 1.3 to 2.9% for the calibration curve of β-myrcene.

Contents (%) of α-pinene and β-myrcene of mastic gum oils of Chios were measured by using the earlier empirical equations (Table 5). According to the proposed method, the mastic gum oils contained 38.1–69.5% α-pinene and 4.5–57.9% β-myrcene. The % RSD fluctuated from 1.5 to 5.3% for α-pinene and 1.2 to 3.1% for β-myrcene.

The large range of α-pinene and β-myrcene percentages was due to the collection time of resin and the duration between its exudation from the trunk and the collection. The concentration of β-myrcene was increased and exceeded α-pinene in resins collected immediately (oil 10), decreased to less than 20%, in resins left to mature physiologically over a maximum time of 2 months (“oil 1”, “oil 2”, “oil 6”, “oil 9”) and was intermediate in other cases: “oil 3”, “oil 4”, “oil 5”, “oil 7”, “oil 8”.

In conclusion, comparison of the GC–MS and FT-Raman methods shows that the results are similar. The quantitative analysis of α-pinene and β-myrcene in gum oil can be determined by FT-Raman spectroscopy. The main advantage of this method over the existing GC–MS method is its simplicity, immediacy, speed and being non-destructive to the sample.

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