

EVALUATION OF A GC-MS METHOD FOR THE ANALYSIS OF OREGANO ESSENTIAL OIL COMPOSITION

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ABSTRACT: The essential oil of oregano (*Origanum heracleoticum* L.) was analyzed and the presence and relative ratio of 33 compounds was successfully determined using a retention time locked gas chromatographic method. Results show that concentration of the injected essential oil plays a very important role in accurate determination of the compounds. While compounds which comprise a small ratio of the total oil (less than 1%) can only be successfully determined when a relatively high amount of oil is injected, higher concentrations can lead to shift in retention times and less efficient separation of the compounds. Lower essential oil concentrations yield better peak shapes and more efficient separation of components, but trace compounds present in a small amount may not be detected or identified with sufficient certainty in these cases. Identification of essential oil components based solely on their chromatographic data and MS spectra is not reliable method of their determination, since essential oils represent very complex mixtures of sometimes highly similar isomeric compounds with similar mass spectra. Retention time locking, which uses matching of spectral data with the locked retention times in a corresponding compound database can be helpful during such analyses, but there is a need for inclusion of much greater number of compounds in such databases. For this reason, calculation of Kovats indices still represents an important step in identification of similar compounds in highly complex natural products such as essential oils.

Keywords: essential oil, oregano, chromatography, GC-MS

INTRODUCTION

Oregano (*Origanum heracleoticum* L.) is a plant native to the Mediterranean region which has been used in traditional medicine and as spice in food for centuries. Its spice value and properties are due to the aromatic and other volatile compounds generally referred to as essential oils of the aromatic plant. Essential oils derived from oregano have valuable pharmacological properties that have been investigated by many scientists around the world (Daferera et al., 2003). Due to their antimicrobial, insecticidal, antifungal, and antibacterial act-

ivities, essential oils have been intensely screened and applied in the fields of pharmacology, medical and clinical microbiology, phytopathology and food preservation (Daferera et al., 2000). Certain essential oils have also been shown to possess cytotoxic effects (Sivropoulou et al., 1996; Bakkali et al., 2008).

Gas chromatography-mass spectrometry (GC-MS) is the most popular method for the determination of essential oil composition. Components existing in the essential oil can be identified by comparison of their

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relative retention indices and their mass spectra (MS). Identification of individual components of essential oils, however, is not always possible using MS data alone. Often different spectra are reported in a library for a single compound, with different common names, or systematic name, corresponding to an individual component sometimes apparent (Sheille et al., 2002). The spectral similarity of a great number of essential oil components causes difficulty in obtaining positive identification of individual components; mass spectra for sesquiterpenes are often identical or nearly identical (Konig et al., 1999). Some authors have also evaluated different techniques for essential oil analysis, like the more comprehensive two-dimensional gas chromatography (GC×GC) (Dimandja et al., 2000; Sheille et al., 2002). However, GC-MS analysis is still the most widely used method for routine analysis of essential oils, and care must be taken to optimize the chromatographic conditions in order to obtain the most accurate results.

The aim of this work was to evaluate the GC-MS method (Agilent application 5988-6530EN, 2010) for the analysis of oregano essential oil and to examine whether calculation of Kovats indices is still a necessary step in identification of the oil components when using a retention time locked chromatographic method. We have also compared the results of analysis of two different concentrations of the oil to optimize the amount of the injected analyte which would obtain the largest number of the identified compounds without the loss of chromatographic resolution.

MATERIALS AND METHODS

Plant materials and chemicals:

n-Heptane of chromatographic grade and was purchased from Merck (Darmstadt, Germany). A mixture of n-alkanes from n-octane (C8) to eicosane (C20) was used for calculation of Kovats indices (KI). Dried and ground plant material of oregano (*Origanum heracleoticum* L.) was obtained from the Institute for Medicinal Plant Research "Dr Josif Pančić" (Belgrade, Serbia).

Isolation of essential oil:

Plant material (50 g of oregano) was subjected to hydrodistillation for 3 h, using a

modified Clevenger-type apparatus to produce essential oil. The oil was dried by anhydrous sodium-sulphate (Na_2SO_4) and kept sealed in dark glass vial at +8 °C until use. Diluted essential oil (1/50 in n-heptane, v/v) was used for GC-MS analyses.

Gas chromatography-mass spectrometry (GC-MS):

GC-MS analyses were carried out using Agilent 5975C Series GC-MSD system (7890A GC and 5975C inert MSD) operating in the EI mode at 70 eV, equipped with a HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.50 µm).

Screener method (Agilent application 5988-6530EN, 2010) using freely available Flavor2 screener compound database for retention time locking (RTL) to n-pentadecane (at 27.500 min) was used for analysis. 1 µl of diluted essential oil was injected in split mode at two different split ratios (25:1 and 12.5:1), and inlet temperature was held at 250 °C. Helium was used as carrier gas in constant pressure mode at 9.4 psi. The oven temperature was programmed as follows: 60 °C raised to 240 °C (3 °C/min) and not held. MSD was operated in scan mode in 40-400 m/z range, with ion source and transfer line temperatures held at 230 and 300 °C, respectively.

Identification of the compounds:

The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with NIST/Flavor2 /Adams libraries spectra and literature (Adams, 1995). ChemStation software (Agilent Technologies) was used for data analysis, and curves used for experimental estimation of Kovats indices were plotted and drawn using SciDaVis

(<http://scidavis.sourceforge.net/>) software.

RESULTS AND DISCUSSION

The results of analysis of oregano essential oil (Table 1.) show that a total of 33 compounds can be determined in oregano oil by the chromatographic method used. Using two split ratios (25:1 and 12.5:1) we achieved formally two different concentrations of injected analytes (dilution of 1/1250 and 1/625, respectively).

Table 1.
Results of the analysis of the oregano essential oil

Compound	RT	Klexp	Kladams	Area % (1:12.5 split ratio)	Area % (1:25 split ratio)
α -pinene	5.22	937.00	937.00	0.86	0.96
camphene	5.58	952.00	953.00	0.22	0.26
1-octen-3-ol	6.32	979.00	978.00	0.42	0.45
3-octanone	6.54	987.00	986.00	0.10	0.10
myrcene	6.68	992.00	991.00	0.66	0.71
α -phellandrene	7.11	1006.00	1005.00	0.10	0.11
δ -3-carene	7.30	1012.00	1011.00	0.07	0.08
α -terpinene	7.50	1019.00	1018.00	0.86	0.94
<i>p</i> -cymene	7.82	1028.00	1026.00	13.59	17.03
limonene	7.91	1031.00	1031.00	0.44	0.48
ocimene	8.18	1040.00	1040.00	0.11	0.11
γ -terpinene	8.93	1062.00	1062.00	1.84	1.97
sabinene hydrate (<i>trans</i>)	9.23	1069.00	1068.00	0.04	n.d.
terpinolene	10.02	1089.00	1088.00	0.15	0.16
linalool	10.44	1099.00	1098.00	0.27	0.29
isoborneol (isomer 2)	13.11	1168.00	1165.00	0.34	0.52
4-carvomenthenol	13.62	1179.00	1177.00	0.86	0.78
α -terpineol	14.16	1191.00	1189.00	0.15	n.d.
dihydro carvone (<i>trans</i>)	14.70	1203.00	1200.00	0.10	n.d.
carvacrol methyl ether	16.48	1246.00	1244.00	0.62	0.61
carvone	17.00	1258.00	1256*	0.11	n.d.
anethole (<i>trans</i>)	18.28	1287.00	1283.00	0.07	n.d.
thymol	18.74	1294.00	1290.00	7.58	7.78
carvacrol	19.51	1309.00	1305**	69.51	83.45
β -caryophyllene	24.08	1419.00	1418.00	1.27	1.28
linalyl-butirate	25.50	1453.00	1450.00	0.19	0.20
γ -muurolene	26.50	1477.00	1477.00	0.05	n.d.
β -bisabolene	27.87	1509.00	1509.00	1.00	n.d.
γ -cadinene	28.06	1514.00	1513.00	0.07	0.15
δ -Cadinene	28.45	1526.00	1524.00	0.15	n.d.
spathulenol	30.63	1578.00	1576.00	0.04	n.d.
caryophyllene oxide	30.83	1582.00	1581.00	0.85	0.86
humulene epoxide II	31.87	1609.00	1606.00	0.11	0.11

* Hognadottir and Rouseff (2003) ** Hennig and Engewald (1994)

Lower concentration of the oil (1:25 split ratio) showed better peak shape and resolution for the separated compounds, but peaks of sabinene hydrate (*trans*), α -terpineol, dihydro carvone (*trans*), carvone, anethole (*trans*), γ -muurolene, γ -cadinene and spathulenol were not present in the chromatogram. Peak of β -bisabolene was

present at this concentration, but could not be determined by comparison with mass spectra data-bases with enough certainty for conclusive determination. Peak at 7.92 min was wrongly identified as terpinyl acetate by comparison with mass spectra data-bases at lower concentration, but using the calculated Kovats index of the peak we

were able to determine the compound in question as limonene, which is in accordance with the findings of other authors (Adam et al., 1998; Bozin et al., 2006).

Chromatogram of the essential oil at higher concentration is given in the Figure 1. Even at this relatively low concentration of essential oil used (dilution of 1/625), it can be seen that the most abundant peaks (of *p*-cymene, thymol and carvacrol) show very large areas compared to other compound

peaks. Peaks of thymol and carvacrol, which are isomeric compounds, show significant difference between their retention times and are satisfactorily separated, but their calculated Kovats indices slightly differ from the theoretical values. Possible reason for this noted difference between theoretical and experimental values of calculated Kovats indices may be a slight shift of retention times of these two compounds due to their relatively high concentrations in the essential oil.

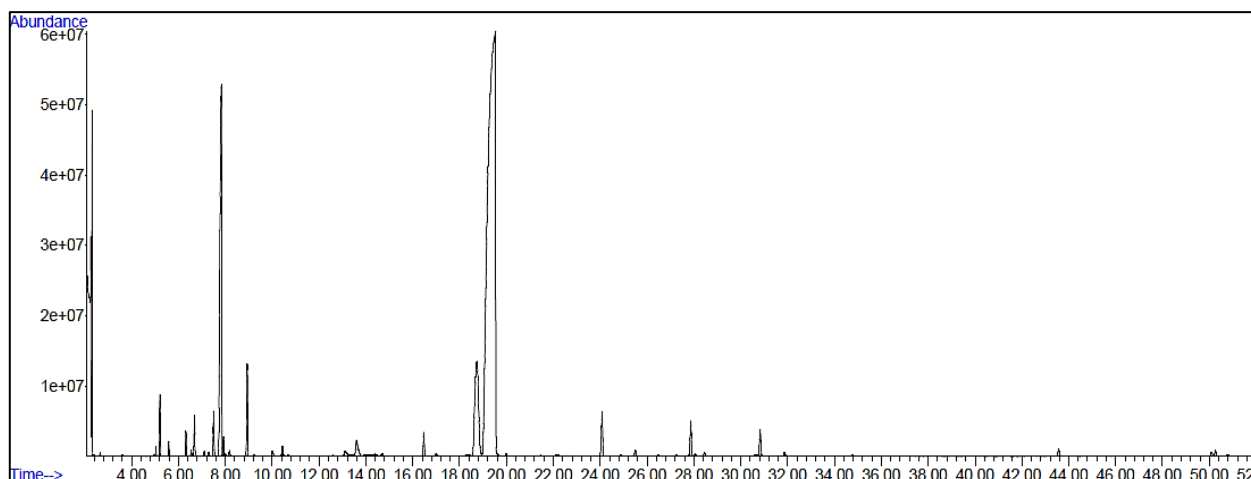


Figure 1. Chromatogram of the oregano essential oil (at 1:12.5 split ratio)

Results show that concentration of the injected essential oil plays a very important role in accurate determination of the compounds. While compounds which comprise a small percent of the total oil (less than 1%) can only be successfully determined when a relatively high amount of oil is injected, higher concentrations can lead to shift in retention times and less efficient separation of the compounds. Lower essential oil concentrations yield better peak shapes and more efficient separation of components, but trace compounds present in a small percentage may not be detected or identified with sufficient certainty in these cases.

The chromatographic method was retention time locked to *n*-pentadecane in the freely available Flavor2 database, which enabled us to make a screener analysis method, which uses matching of spectral data with the locked retention times in the corresponding screener Flavor2.SCR database. The used method also had a relatively short

run time of 60 minutes when compared to some other GC-MS methods used for analysis of essential oils (Mimica-Dukić et al., 2003; Bozin et al., 2006). Chromatogram was analyzed using the screener analysis method, but due to relatively small number of compound entries in the database (about 400), only a small number of compounds was determined, and those results were not included in this paper.

CONCLUSION

Referring to the obtained results, screener method (Agilent application 5988-6530EN, 2010) using freely available Flavor2 database can be used for the analysis of oregano essential oil composition, but due to the small number of entries in the database and shift of retention times in the case of most abundant compounds, calculation of Kovats indices still represents an important step in identification of similar compounds in highly complex natural products such as essential oils.

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REFERENCES

1. Adam K., Sivropoulou A., Kokkini S., Lanaras T., Arsenakis M. (1998), Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi, *J. Agric. Food Chem.*, **46**, 1739-1745.
2. Adams RP. (1995), Identification of essential oil components by gas chromatography / mass spectroscopy, *Allured Publishing Corporation*, Carol Stream: Illinois, USA.
3. Bakkali F., Averbeck S., Averbeck D., Idomar M. (2008), Biological effects of essential oils – a review, *Food and Chemical Toxicology*, **46**, 446–475.
4. Bozin B., Mimica-Dukic N., Simin N., Anakov G. (2006), Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils, *J. Agric. Food Chem.*, **54**, 1822-1828.
5. Hennig P., Engewald W. (1994), Influence of adsorption effects on retention indices of selected C10-hydroxy compounds at various temperatures, *Chromatographia*, **38** (1/2), 93-97.
6. Daferera D.J., Ziogas B.N., Polissiou M.G. (2000), GC–MS analysis of essential oils from some greek aromatic plants and their fungitoxicity on *Penicillium digitatum*, *Journal of Agricultural Food Chemistry*, **48**, 2576–2581.
7. Daferera D. J., Ziogas B. N., Polissiou, M. G. (2003), The Effectiveness of plant essential oils on *Botrytis cinerea*, *Fusarium* sp., and *Clavibacter michiganensis* subsp. *Michiganensis*, *Crop Prot.*, **22**, 39–44.
8. David F., Scanlan F., Sandra P., Szelewski M. (2010), Analysis of essential oil compounds using retention time locked methods and retention time databases, Application, *Agilent Technologies*, 5988-6530EN.
9. Dimandja J.-M., Stanfill S., Grainger J., Patterson D. Jr. (2000), Application of comprehensive two-dimensional gas chromatography (GC/GC) to the qualitative analysis of essential oils, *J. High Resol. Chromatogr.*, **23**, 208-214.
10. Hognadottir A., Rouseff R. (2003), Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry, *Journal of Chromatography A*, **998**, 201–211.
11. Konig W.A., Bulow N., Saritas Y. (1999), Identification of sesquiterpene hydrocarbons by gas phase analytical methods, *Flavour Fragr. J.*, **14**, 367-378.
12. Mimica-Dukić N., Kujundžić S., Soković M., Couladis M. (2003), Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill. obtained by different distillation conditions, *Phytother. Res.*, **17**, 368–371.
13. Sheille R., Mondello L., Marriott P., Dugo G. (2002), Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography, *Journal of Chromatography A*, **970**, 225–234.
14. Sivropoulou A., Papanikolaou E., Nikolaou C., Kokkini S., Lanaras T., Arsenakis M. (1996), Antimicrobial and cytotoxic activities of *Origanum* essential oils, *J. Agric. Food Chem.*, **44**, 1202-1205.

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