Development and Validation of a GC-MS/MS Method for the Simultaneous Quantification of Selected Compounds in Essential Oils

https://doi.org/10.15255/KUI.2024.023
KUI-40/2024
Original scientific paper
Received June 27, 2024
Accepted August 17, 2024

A. Vučak, *G. Golubić*, and *F. Burčul* *
University of Split, Faculty of Chemistry and Technology, Ruđera Boškovića 35, 21 000 Split, Croatia

Abstract

The benefits from the everyday use of essential oils are associated with well-known antimicrobial, anti-inflammatory, and antioxidant activity of their constituents. Considering this, compounds within essential oils are the focus of extensive research, necessitating the development of analytical methods for their simple and rapid determination. In this study, a method for the quantitation of *p*-cymene, limonene, eucalyptol, linalool, menthol, and carvone in essential oils was developed and validated.

Gas chromatography-tandem mass spectrometry (GC-MS/MS) was employed for the separation and determination of the selected compounds. Standard solutions of six analytes were prepared using hexane as the solvent. A triple quadrupole mass spectrometer, operated in dMRM scan mode, was used to monitor specific transitions at their optimal collision energies (quantifier and two qualifiers for each compound). Gas chromatography was optimised by adjusting the oven temperature gradient programme to achieve efficient separation in a short run time of 14 min.

All calibration curves showed good linearity ($R^2 \geq 0.998$), with the concentration ranges varying depending on the analyte (0.10–10.00 μg ml^{-1}). The method was validated for accuracy (80.23–115.41 %), intra-day precision (≤ 12.03 %), and inter-day precision (≤ 11.34 %). The validation followed ICH guidelines, and all tested parameters were found to be satisfactory. This confirms the method's suitability for the simultaneous individual determination of these compounds in essential oils. The method was successfully applied in the analysis of essential oils from lemon, tangerine, grapefruit, eucalyptus, myrtle, niaouli, peppermint, and fennel.

Keywords

Essential oils, GC-MS/MS, triple quadrupole, MRM transition, validation

1 Introduction

Essential oils are complex mixtures of volatile compounds extracted from plant raw material through methods such as hydrodistillation, steam distillation, or dry distillation. Additionally, mechanical processes like cold pressing are used for citrus fruits to preserve the thermosensitive constituents.[1] Produced and stored in various secretory structures, essential oils differ in chemical composition and biological function, playing a crucial role in plant defence and signalling systems in the natural world.[1,2] Due to their well-known biological activities (antimicrobial, antioxidant, anti-inflammatory, anticancer), essential oils offer numerous benefits when used in everyday applications.[3] In addition, essential oils are characterized by strong aromas, making them popular additives in cosmetics and foods, as well as effective botanical insecticides.[2,3] The main group of compounds found in essential oils are monoterpenes and sesquiterpenes, with phenylpropanoids frequently present.[1] *p*-Cymene (1-methyl-4-(propan-2-yl)benzene) is a monocyclic monoterpene with a fresh, woody aroma, known for its antibacterial, anticancer, calming, painkilling, and anti-inflammatory properties.[5] Limonene (1-methyl-4-(prop-1-en-2-yl)cyclohexene) is a common component of many essential oils, strongly abundant in essential oils from citrus fruit peels. Its pleasant lemon-like aroma, combined with its widespread occurrence, makes this monocyclic monoterpene a frequent and inexpensive fragrance ingredient in cosmetic products.[6]

Eucalyptol or 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), bicyclic monoterpenoid ether, is the primary compound in eucalyptus essential oil and the fresh aroma compound in essential oils from other Myrtaceae plants. High amounts of eucalyptol are also present in tea trees and sage.[1,7] Eucalyptol is a frequent ingredient in toothpaste and mouthwash due to its cooling taste, and it also serves as a cough suppressant.[1] There are many therapeutic applications of eucalyptol across multiple diseases.[8] Linalool (3,7-dimethyl-octa-1,6-dien-3-ol), an acyclic monoterpenoid alcohol, is a key compound in several essential oils, such as lavender, bay laurel, sweet basil, coriander, and sweet orange.[1] Like limonene, linalool

* Corresponding author: Assoc. Prof. Franko Burčul, PhD
e-mail: franko@ktf-split.hr

is a common ingredient in cosmetics due to its floral aroma with a slight citrus note. However, both linalool and limonene are declared as allergens.[9] In addition, linalool plays a significant role in the phytochemical activities of lavender essential oil derived from the flower heads.[1] Menthol (5-methyl-2-(propan-2-yl)cyclohexan-1-ol), a monocyclic monoterpenoid alcohol, is a minty and cooling compound present in several Mentha species. It is used as a flavouring agent in chewing gum and toothpaste, as well as in cosmetic products, cough medicines, and topical analgesics.[1] Menthol is also known for its antibacterial, antifungal, antipruritic, anticancer, and analgesic properties.[10] Carvone (2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one) is a minty, herbaceous monocyclic monoterpenoid ketone found in caraway seed, dill oils, spearmint, eucalyptus, and mandarin.[1] The chemical structures of these compounds are presented in Fig. 1.

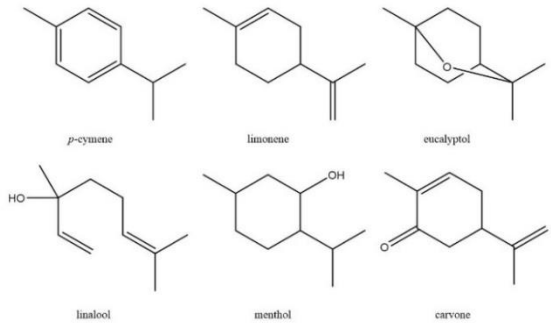

Fig. 1 – Chemical structures of the analysed compounds
Slika 1 – Kemijske strukture analiziranih spojeva

Given the extensive research on biologically active compounds in essential oils, there is a need for improved analytical methods that enable their simple and rapid quantification. The preferred technique for analysing volatile compounds is gas chromatography coupled with a flame ionisation detector (GC-FID) or a mass spectrometer (GC-MS).[11] Additionally, high-performance liquid chromatography with a diode array detector (HPLC-DAD) or HPLC-MS can be applied.[12] Various approaches exist for the determination of *p*-cymene,[5,13,14] limonene,[15,16] linalool,[16,17] and eucalyptol.[18,19] For pharmacokinetic studies in rats, *Sa et al.*[18] and *Hou et al.*[19] developed GC-MS/MS methods in multiple reaction monitoring (MRM) mode, focusing on improving sensitivity and selectivity. However, for essential oil constituents, such methods are scarce in the literature. The aim of this study was to develop and validate a simple and rapid GC-MS/MS method based on MRM transitions for the simultaneous quantitative determination of *p*-cymene, limonene, eucalyptol, linalool, menthol, and carvone. To the best of our knowledge, this is the first method to achieve simultaneous quantification of these six compounds. The method does not require derivatisation, and sample preparation for essential oils involves only dissolving in hexane. Moreover, the method is highly sensitive, and can determine concentrations typically starting from 0.50 μg ml^{-1}. In the case of carvone, it starts even lower, from 0.10 μg ml^{-1}. While these compounds are abundant in essential oils, the proposed method can be adapted for their determination in other samples, such as cosmetic products and biological samples.

2 Experimental

2.1 Chemicals and reagents

The standards of *p*-cymene (purity > 99.5 %), limonene (purity ≥ 99.0 %), eucalyptol (purity ≥ 99.0 %), linalool (purity 97 %), menthol (purity 99 %) and carvone (purity ≥ 99.0 %) were purchased from Sigma Aldrich (St. Louis, USA). Analytes used in this study were liquids, except for solid menthol, all being well-soluble and stable in hexane. Accordingly, hexane (purity ≥ 97 %) was selected as a solvent and obtained from VWR International (Wien, Austria). For real sample analysis, essential oils were purchased as follows: lemon, tangerine, grapefruit, and niaouli from Pranarōm (Ghislenghien, Belgium), eucalyptus from N-Elements (Sveta Nedelja, Croatia), myrtle from Dea Flores (Rijeka, Croatia), peppermint and fennel from Aromara (Harmica, Croatia).

2.2 Stock solutions, calibration solutions and quality control solutions

To prepare the stock solution for each compound, 50 μl of the standard was precisely weighed and dissolved in hexane to a final volume of 10.0 ml using volumetric flask. The concentration of each stock solution was approximately 5 mg ml^{-1}, with the exact concentration calculated based on the mass of the added analyte. The working standard solutions were prepared by diluting the appropriate volume (based on the exact concentration) of each stock solution with hexane to achieve a final concentration of 50 μg ml^{-1} for each analyte. The stock solutions were stored at −20 °C until use, while working solutions were prepared daily. The calibration solutions were prepared in triplicate (n = 3) by diluting the working standard in hexane to obtain concentrations of 0.10, 0.25, 0.50, 0.75, 1.00, 2.50, 5.00, 7.50, and 10.0 μg ml^{-1}. The quality control (QC) solutions were prepared in the same way as the calibration solutions to achieve LLOQ (low limit of quantitation), low, medium, and high QC concentrations.

2.3 GC-MS/MS instrument and conditions

All analyses were conducted using an 8890 GC system equipped with 7693A autosampler, coupled to a triple

quadrupole mass spectrometer 7000D GC/TQ (Agilent, Santa Clara, CA, USA). Chromatographic separation was achieved on a nonpolar (5 %-phenyl)-methylpolysiloxane capillary column HP-5MS (30 m × 0.25 mm i.d. and 0.25 μm film thickness; Agilent, Santa Clara, CA, USA). Helium (grade 5.0) was used as the carrier gas, with a constant column flow of 1.0 ml min^{-1}. Using the autosampler, 1 μl of the sample was injected with a standard injection type in split inlet mode (50 : 1) at an inlet temperature of 250 °C. The oven temperature gradient programme was optimised for rapid and efficient separation. The initial column temperature of 70 °C was held for 3 min. Firstly, the temperature was slowly ramped to 100 °C at 5 °C min^{-1} with a hold time of 1 minute, and further ramped to 246 °C at 120 °C min^{-1} and held for 3 min. The MS transfer line temperature was set at 280 °C. The mass spectrometer operated using electron ionisation at 70 eV, with an ion source temperature of 230 °C. Nitrogen (grade 5.0) was used as the collision gas (1.5 ml min^{-1}) for MS/MS fragmentations, with He as the quench gas (2.5 ml min^{-1}). The MRM transitions (quantifier and two qualifiers) with corresponding collision energies (CEs) were optimised for each compound. The selected scan type was dynamic multiple reaction monitoring (dMRM). The method run time was 14 min, including a 3 min solvent delay (during which the solvent was eluting and mass spectra was not being recorded). The acquired data were analysed using MassHunter Workstation Software (version 10.0, Agilent, Santa Clara, CA, USA).

2.4 Method validation

Method validation was performed in accordance with ICH Q2(R2) and ICH M10 guidelines for the validation of analytical procedures.[20,21] Linearity was tested by analysing the calibration solutions at 9 concentration levels over the concentration range of 0.10–10.00 μg ml^{-1}. The solutions were run from low to high concentrations. Each level was measured three times, and the average signal was plotted against concentration. The concentration range for each analyte was selected considering linearity and accuracy of the measured concentrations. Limits of detection and quantitation were determined based on a signal-to-noise ratio (S/N) of 3 for LOD and 10 for LOQ. Accuracy was calculated by comparing measured concentrations to those calculated from the calibration curve equation. Accuracy was determined for both calibration and quality control solutions and expressed as relative error (%RE). Precision was calculated for the quality control solutions (LLOQ, low, medium, and high QCs) as relative standard deviation (%RSD). Intra-day precision was measured by analysing three replicates of each QC prepared on the same day under the same conditions. Inter-day precision was measured by analysing QCs prepared on three consecutive days, while other parameters remained unchanged. The concentration of LLOQ was equal to the lowest concentration level, while LQC concentration was three times higher than LLOQ (0.30 μg ml^{-1} for carvone unlike 1.50 μg ml^{-1} for other compounds). The medium and high QC concentrations should be 30 – 50 % and above 75 % of the concentration curve range.[15] Hence, the selected MQC and HQC concentrations for all compounds were 3.00 and 7.50 μg ml^{-1}, respectively.

3 Results and discussion

3.1 Method development

The study aimed to develop a simple, rapid, and reliable GC-MS/MS method for the simultaneous determination of six selected compounds commonly found in essential oils. To achieve this, optimisations were carried out for both the gas chromatography and mass spectrometry parameters.

3.1.1 MS/MS Optimisation

Initially, individual solutions of each compound dissolved in hexane were analysed in full scan MS mode. The mass spectrum that agreed with the mass spectra from NIST17 and Wiley9N08 databases was obtained for each compound. Based on these spectra, two fragments with the highest detector responses were selected as precursor ions for each compound. Next, in single ion monitoring (SIM) mode, each precursor ion was fragmented with nitrogen in collision-induced dissociation (CID) at 15, 30, 45, and 60 eV. The product ions were selected as the highest signals from the obtained spectra on all four collision energies. Subsequently, all specific transitions of precursor ions to product ions were monitored in multiple reaction monitoring (MRM) mode across 30 different collision energies (ranging from 2 to 60 eV in increments of 2 eV) to determine the optimum collision energy for each MRM transition. The MRM transitions of the precursor ions with the highest detector responses were then optimised regarding their product ions and corresponding collision energies. Finally, by simultaneously monitoring the transitions associated with each compound, the quantifier was selected as the highest signal in the MS/MS spectrum. Two other transitions with significant detector responses were selected as qualifiers to confirm the presence of the compound. In the literature, it is recommended to use a quantifier and at least one qualifier.[22,23] The MRM transitions with the optimum collision energies together with retention times for all compounds are listed in Table 1.

3.1.2 GC Optimisation

Gas chromatography was optimised to achieve efficient separation in a short run time. The initial method, developed according to *Adams*[24], followed this oven temperature programme: an initial hold at 60 °C for 3 min, ramp to 246 °C at 3 °C min^{-1} and hold for 25 min. To reduce the method run time, various gradient programmes with more than one temperature ramp were tested. Given the close retention times of *p*-cymene, limonene, and eucalyptol, a slower temperature ramp was used to achieve their chromatographic separation. Otherwise, the maximum temperature rate (120 °C min^{-1}) was used. The final oven temperature programme was as follows: an initial hold at 70 °C for 3 min, ramp to 100 °C at 5 °C min^{-1}, hold for 1 min, then ramp to 246 °C at 120 °C min^{-1} and hold for 3 min. Thus, the run time was reduced from 89 to 14 min. The final chromatogram of the six compounds is presented in Fig. 2.

Table 1 – Retention times (*RT*) and MRM transitions with the optimum collision energies (*CE*)
Tablica 1 – Retencijska vremena (*RT*) i MRM prijelazi s optimalnim energijama sraza (*CE*)

Compound	*RT* / min	Quantifier		Qualifier 1		Qualifier 2	
		Transition (*m*/*z*)	*CE* / eV	Transition (*m*/*z*)	*CE* / eV	Transition (*m*/*z*)	*CE* / eV
p-cymene	7.05	119 → 91	12	119 → 77	24	134 → 119	6
limonene	7.16	93 → 77	14	93 → 51	34	68 → 53	10
eucalyptol	7.24	108 → 93	6	108 → 77	24	139 → 43	16
linalool	9.06	93 → 77	12	93 → 51	36	71 → 43	6
menthol	10.62	95 → 67	8	81 → 79	12	81 → 41	20
carvone	11.18	82 → 39	10	108 → 77	22	108 → 93	6

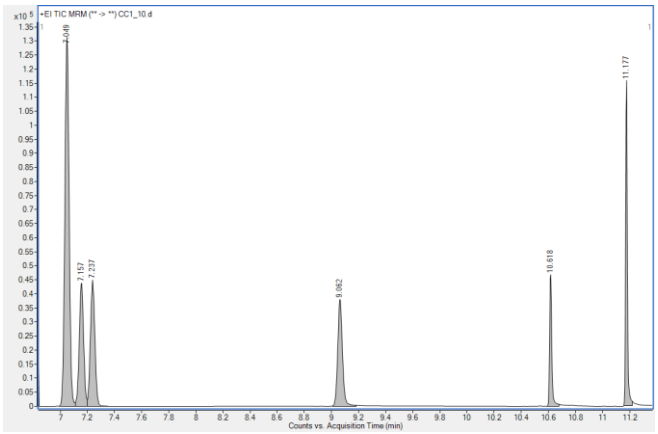

Fig. 2 – Chromatogram of *p*-cymene, limonene, eucalyptol, linalool, menthol, and carvone

Slika 2 – Kromatogram *p*-cimena, limonena, eukaliptola, linalola, mentola i karvona

3.2 Method validation

The parameters tested for this purpose were specificity, accuracy, precision, linearity, limit of detection, and limit of quantification.

3.2.1 Specificity

To unequivocally identify the analyte in the presence of other components that may be found in the sample (impurities, degradants, matrix), it is necessary to ensure the specificity of the analytical procedure. When using mass spectrometry, specificity can be ensured and predicted through technical parameters, making experimental studies unnecessary.[20] Furthermore, technology inherent justification is supported using MRM transitions in combination with retention times, as specified in Table 1. Each compound was defined by the combination of retention time, quantifier, two qualifiers, and their corresponding collision energies, as well as by the distinct constant ratios between quantifier and qualifiers. Although *p*-cymene, limonene, and eucalyptol have close retention times, their determination is ensured based on different quantifiers and collision energies. On the other hand, limonene and linalool both have quantifier 93 → 77 at close collision energies (14 and 12 eV, respectively), and one same qualifier (93 → 51 on 34 and 36 eV, respectively), but the second qualifiers are different, and these two compounds are well separated. Therefore, the interferences that may be present were "filtered" out by using MRM mode.

3.2.2 Range, linearity, LOD and LOQ

A linear regression model without weighing was found to provide a good data fit for all analytes and was selected as the most appropriate model for these compounds. The calibration curve equations, along with their basic characteristics are shown in Table 2. Most compounds had a calibration curve in the range of 0.50–10.00 μg ml^{-1}. In contrast, the highest concentration level for menthol and carvone calibration curves was 7.50 μg ml^{-1}. In addition, carvone had the lowest starting calibration curve level (0.10 μg ml^{-1}). All calibration curves showed very good linearity with a correlation coefficient ≥ 0.998. Limits of detection and quantitation were calculated as a sufficient amount of analyte that must be present in the sample to produce a signal that can be reliably distinguished from the noise. The lowest concentration level of each compound was repeatedly measured to obtain average signal-to-noise ratios. LOD and LOQ values were always below the first calibration level, also shown in Table 2.

3.2.3 Accuracy and precision

The acceptable intervals for relative error and RSD, as per ICH Q2(R2) and ICH M10 guidelines, are ± 20 % for *LLOQ* and ± 15 % for low, medium, and high QCs.[15,20,21] Accuracy values over the entire calibration range were from 80.23 to 115.41 % (Table 2), which is in accordance with the established criteria. Accuracy and precision (both intra- and inter-day) of the developed method were evaluated by analyses of quality control solutions, while the results are summarised in Table 3. Intra-day imprecision and inaccuracy ranged from 0.32 to 12.03 %, and from 0.08 to 12.61 %, respectively. Inter-day imprecision and inaccuracy ranged from 1.09 to 11.34 %, and from 0.63 to 19.84 %, respectively. The maximum RSDs were obtained for menthol and carvone (12.03 %) in the intra-day assay, and for linalool (11.34 %) in the inter-day assay. Except for MQC for menthol (*RE* = 16.07 %) and carvone (*RE* = 19.84 %), all results were within the acceptable interval. Thus, the developed method is sufficiently accurate, reproducible, and precise.

3.3 Essential oils analysis

Finally, the method was tested in the analysis of essential oils. Eight selected essential oils were weighed and diluted in hexane to obtain analyte concentrations within the method linear range. The results are presented in Table 4 as weight concentrations (% *w*/*w*) of compounds in essential oils, and a chromatogram of each sample is given in the supplementary material Figs. S1 and S2. Limonene was the most abundant in grapefruit (*Citrus x paradisi* Macfad), tangerine (*Citrus reticulata* Blanco), and lemon (*Citrus limon* L.), while high percentages of eucalyptol were found in eucalyptus (*Eucalyptus globulus* Labill.), niaouli (*Melaleuca quinquenervi*a (Cav.) S.T. Blake), and myrtle (*Myrtus communis* L.). The presence of *p*-cymene was observed in all samples (significant amounts in tangerine and lemon). Minute amounts of linalool and carvone were quantified in all essential oils. Menthol was quantified in peppermint (*Mentha x piperita* L.), and detected in eucalyptus and fennel (*Foeniculum vulgare* Mill.).

Table 2 – Ranges and analytical figures of merit for the calibration curve
Tablica 2 – Rasponi i analitičke vrijednosti krivulja umjeravanja

Compound	Range / μg ml^{-1}	*n*	Calibration curve equation	R^2	*RSD* / %	Accuracy / %	*LOD* / μg ml^{-1}	*LOQ* / μg ml^{-1}
p-cymene	0.50–10.00	7	$y = 13638x - 2633$	0.998	6.38	89.19–109.26	0.07	0.23
limonene	0.50–10.00	7	$y = 4509x - 750$	0.998	7.74	88.16–112.61	0.13	0.44
eucalyptol	0.50–10.00	7	$y = 3585x - 499$	0.998	5.04	91.25–105.02	0.14	0.45
linalool	0.50–10.00	7	$y = 3653x + 863$	0.998	9.37	82.80–110.50	0.10	0.34
menthol	0.50–7.50	6	$y = 2095x + 204$	0.998	9.70	80.23–107.53	0.09	0.28
carvone	0.10–7.50	8	$y = 5933x - 141$	0.999	8.76	83.78–115.41	0.02	0.07

n – number of points in calibration curve

4 Conclusion

Gas chromatography-tandem mass spectrometry (GC-MS/MS) is an effective technique for the quantitation of volatile constituents in essential oils. In this study, a simple and rapid method for the determination of *p*-cymene, limonene, eucalyptol, linalool, menthol, and carvone in essential oils was developed and optimised. The optimised oven temperature programme enabled the separation of these six compounds in less than 5 min. Quantification of each compound was based on the main MRM transition with two additional MRM transitions used for the

confirmation of each compound, ensuring specificity. The method demonstrated very good linearity, low limits of detection and quantification, and was validated according to international guidelines for accuracy, intra-day precision, and inter-day precision. This procedure was successfully applied in the analysis of eight commercial essential oils. Moreover, the method can be further modified for the analysis of additional analytes of interest with similar properties. Compared to techniques like liquid chromatography, gas chromatography is more environmentally friendly, making GC-MS/MS an increasingly popular choice for analytical methods like the one presented in this study.

Table 3 – Evaluation of accuracy and precision based on LLOQ, low, medium and high QCs (3 replicates per day, and 3 replicates for 3 days)

Tablica 3 – Procjena točnosti i preciznosti na temelju kontrolnih otopina (3 ponavljanja u jednom danu i 3 tijekom 3 dana)

Compound	Nominal concentration / μg ml^{-1}	Intra-day assay			Inter-day assay		
		Measured concentration mean ± SD / μg ml^{-1}	*RSD* / %	*RE* / %	Measured concentration mean ± SD / μg ml^{-1}	*RSD* / %	*RE* / %
p-cymene	0.50	0.53 ± 0.01	1.46	5.69	0.56 ± 0.02	4.45	11.01
	1.50	1.57 ± 0.11	7.25	4.61	1.65 ± 0.09	5.36	10.07
	3.00	3.00 ± 0.04	1.48	0.13	3.30 ± 0.18	5.43	9.88
	7.50	7.21 ± 0.29	4.00	3.92	7.57 ± 0.47	6.27	0.93
limonene	0.50	0.50 ± 0.01	2.99	0.45	0.56 ± 0.02	4.22	12.42
	1.50	1.52 ± 0.10	6.39	1.59	1.71 ± 0.07	4.16	13.75
	3.00	2.99 ± 0.04	1.49	0.28	3.35 ± 0.08	2.49	11.52
	7.50	7.45 ± 0.02	0.32	0.61	6.78 ± 0.07	1.09	9.58
eucalyptol	0.50	0.51 ± 0.02	3.00	1.31	0.50 ± 0.04	7.04	0.63
	1.50	1.68 ± 0.02	1.44	11.83	1.61 ± 0.14	8.67	7.59
	3.00	3.04 ± 0.10	3.14	1.24	3.10 ± 0.23	7.35	3.41
	7.50	7.51 ± 0.08	1.11	0.08	7.64 ± 0.49	6.46	1.90
linalool	0.50	0.50 ± 0.04	8.22	0.90	0.41 ± 0.05	11.34	18.03
	1.50	1.60 ± 0.04	2.56	6.50	1.55 ± 0.13	8.33	3.16
	3.00	2.62 ± 0.12	4.75	12.61	2.88 ± 0.31	10.71	3.92
	7.50	7.38 ± 0.16	2.23	1.55	7.58 ± 0.49	6.48	1.03
menthol	0.50	0.51 ± 0.02	4.72	2.52	0.45 ± 0.05	10.48	10.43
	1.50	1.51 ± 0.10	6.78	0.35	1.43 ± 0.10	7.20	4.97
	3.00	2.92 ± 0.35	12.03	2.60	2.52 ± 0.16	6.26	16.07
	7.50	6.71 ± 0.31	4.69	10.52	6.95 ± 0.55	7.89	7.38
carvone	0.10	0.11 ± 0.003	2.42	8.06	0.10 ± 0.01	10.74	3.41
	0.30	0.29 ± 0.02	5.55	3.69	0.28 ± 0.02	8.05	6.55
	3.00	2.77 ± 0.33	12.03	7.62	2.40 ± 0.13	5.54	19.84
	7.50	7.26 ± 0.28	3.83	3.24	6.69 ± 0.24	3.53	10.86

Table 4 – Results of essential oils analysis
Tablica 4 – Rezultati analize eteričnih ulja

Essential oil	*p*-Cymene / % *w/w*	Limonene / % *w/w*	Eucalyptol / % *w/w*	Linalool / % *w/w*	Menthol / % *w/w*	Carvone / % *w/w*
lemon	3.21 ± 0.11	45.69 ± 1.28	–	0.22 ± 0.01	–	0.059 ± 0.004
tangerine	4.32 ± 0.15	64.78 ± 1.81	–	0.26 ± 0.01	–	0.012 ± 0.001
grapefruit	1.42 ± 0.05	92.20 ± 2.58	–	0.44 ± 0.02	–	0.322 ± 0.019
eucalyptus	1.55 ± 0.06	2.37 ± 0.07	42.55 ± 0.92	< 0.02	< 0.01	0.012 ± 0.001
myrtle	0.36 ± 0.01	4.12 ± 0.12	22.79 ± 0.50	2.10 ± 0.09	–	0.077 ± 0.005
niaouli	0.86 ± 0.03	2.82 ± 0.08	43.25 ± 0.94	0.12 ± 0.01	–	0.012 ± 0.001
peppermint	0.16 ± 0.01	2.76 ± 0.08	6.48 ± 0.14	0.13 ± 0.01	28.43 ± 2.01	0.057 ± 0.003
fennel	1.06 ± 0.04	3.01 ± 0.08	< 0.45	0.060 ± 0.003	< 0.02	0.042 ± 0.003

Data are expressed as mean value ± RSD (n = 3); – not detected

ACKNOWLEDGEMENTS

"Functional integration of the University of Split, PMF-ST, PF-ST, and KTF-ST through the development of scientific research infrastructure in the building of three faculties" (KK.01.1.1.02.0018), a project co-financed by the Croatian Government and the European Union through the European Regional Development Fund – the Competitiveness and Cohesion Operational Programme.

List of abbreviations and symbols
Popis kratica i simbola

CE – collision energy
CID – collision-induced dissociation
DAD – diode array detector
dMRM – dynamic multiple reaction monitoring
FID – flame ionisation detector
GC-MS/MS – gas chromatography-tandem mass spectrometry
HPLC – high-performance liquid chromatography
HQC – high concentration quality control solution
ICH – International Council for Harmonisation
i.d. – inner diameter
LLOQ – low limit of quantitation
LOD – limit of detection
LOQ – limit of quantitation
LQC – low concentration quality control solution
m/z – mass-to-charge ratio
MRM – multiple reaction monitoring
MQC – medium concentration quality control solution
NIST – National Institute of Standards and Technology
R^2 – correlation coefficient
RE – relative error
RSD – relative standard deviation
RT – retention time
S/N – signal-to-noise ratio
SD – standard deviation
SIM – single ion monitoring
QC – quality control solution
VOCs – volatile organic compounds

References
Literatura

1. *M. Zuzarte, L. Salgueiro,* Essential Oils Chemistry, in D. de Sousa (eds), Bioactive Essential Oils and Cancer. Vol. 2, Springer, Cham, 2015, pp. 19–61.
2. *C. Bowsher, M. Steer, A. Tobin,* Terpenoids, in E. Owen (eds), Plant Biochemistry. Vol. 12, Garland Science, New York, 2008.
3. *A. Masyita, R. Mustika Sari, A. Dwi Astuti, B. Yasir, R. N, Rahma Rumata, T. B. Emran, F. Nainu, J. Simal-Gandara,* Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives, Food Chem.: X. **13** (2022) 100217, doi: https://doi.org/10.1016/j.fochx.2022.100217.
4. *J. B. Sharmeen, F. M. Mahomoodally, G. Zengin, F. Maggi,* Essential Oils as Natural Sources of Fragrance Compounds for Cosmetics and Cosmeceuticals, Molecules **26** (2021) 666, doi: https://doi.org/10.3390/molecules26030666.
5. *G. A. Shabir,* Method development and validation for the GC-FID assay of *p*-cymene in tea tree oil formulation, J. Pharm. Biomed. Anal. **39** (2005) 681–684, doi: https://doi.org/10.1016/j.jpba.2005.03.036.
6. *A. J. Vieira, F. P. Beserra, M. C. Souza, B. M. Totti, A. L. Rozza,* Limonene: Aroma of innovation in health and disease, Chem.-Biol. Interact. **283** (2018) 97–106, doi: https://doi.org/10.1016/j.cbi.2018.02.007.

7. *Z. M. Cai, J. Q. Peng, Y. Chen, L. Tao, Y. Y. Zhang, L. Y. Fu, Q. D. Long, X. C. Shen,* 1,8-Cineole: a review of source, biological activities, and application, J. Asian Nat. Prod. Res. **10** (2020) 938–954, doi: https://doi.org/10.1080/10286020.2020.1839432.
8. *C. C. Hoch, J. Petry, L. Griesbaum, T. Weiser, K. Werner, M. Ploch, A. Verschoor, G. Multhoff, A. Bashiri Dezfouli, B. Wollenberg,* 1,8-cineole (eucalyptol): A versatile phytochemical with therapeutic applications across multiple diseases, Biomed. Pharmacother. **167** (2023) 115467, doi: https://doi.org/10.1016/j.biopha.2023.115467.
9. *A. Sarkic, I. Stappen,* Essential Oils and Their Single Compounds in Cosmetics—A Critical Review, Cosmetics **5** (2018) 11, doi: https://doi.org/10.3390/cosmetics5010011.
10. *G. P. Kamatou, I. Vermaak, A. M. Viljoen, B. M. Lawrence,* Menthol: a simple monoterpene with remarkable biological properties, Phytochemistry **96** (2013) 15–25, doi: https://doi.org/10.1016/j.phytochem.2013.08.005.
11. *G. B. Lockwood,* Techniques for gas chromatography of volatile terpenoids from a range of matrices, J. Chromatogr. A **936** (2001) 23–31, doi: https://doi.org/10.1016/S0021-9673(01)01151-7.
12. *C. Turek, F. C. Stintzing,* Application of high-performance liquid chromatography diode array detection and mass spectrometry to the analysis of characteristic compounds in various essential oils, Anal. Bioanal. Chem. **400** (2011) 3109–3123, doi: https://doi.org/10.1007/s00216-011-4976-5.
13. *X. Hu, Y. Chu, G. Ma, W. Li, X. Wang, H. Mo, Q. Yin, J. Guo, X. Ma, S. Zhou,* Simultaneous determination of ascaridole, *p*-cymene and α-terpinene in rat plasma after oral administration of Chenopodium ambrosioides L. by GC-MS, Biomed. Chromatogr. **29** (2015) 1682–1686, doi: https://doi.org/10.1002/bmc.3479.
14. *J. Bisht, A. Khristi,* Development and Validation of Novel High-Performance Liquid Chromatography Method for Simultaneous Estimation of *p*-Cymene and Aloe-emodin, J. Nat. Rem. **23** (2023) 185–190, doi: https://doi.org/10.18311/jnr/2023/31612.
15. *M. Son, J. Yang, S. Cho, J. Lee, H. B. Oh,* GC–MS Method for the Quantitative Analysis of Limonene from Genetically Engineered Saccharomyces cerevisiae, Bull. Korean Chem. Soc. **39** (2018) 1368–1372, doi: https://doi.org/10.1002/bkcs.11607.
16. *J. Rudbäck, N. Islam, U. Nilsson, A. T. Karlberg,* A sensitive method for determination of allergenic fragrance terpene hydroperoxides using liquid chromatography coupled with tandem mass spectrometry, J. Sep. Sci. **36** (2013) 1370–1378, doi: https://doi.org/10.1002/jssc.201200855.
17. *Y. H. Wang, G. Mondal, N. Stevens, C. Bascoul, R. J. Osguthorpe, I. A. Khan, C. R. Yates,* Development of a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for Characterizing Linalool Oral Pharmacokinetics in Humans, Molecules **28** (2023) 6457, doi: https://doi.org/10.3390/molecules28186457.
18. *C. Sa, J. Liu, Y. Dong, L. Jiang, G. Gentana, A. Wurita,* Quantification of eucalyptol (1,8-cineole) in rat serum by gas chromatography-mass/mass spectrometry and its application to a rat pharmacokinetic study, Biomed. Chromatogr. **35** (2021) e5080, doi: https://doi.org/10.1002/bmc.5080.
19. *M. Z. Hou, L. L. Chen, C. Chang, J. F. Zan, S. M. Du,* Pharmacokinetic and tissue distribution study of eight volatile constituents in rats orally administrated with the essential oil of Artemisiae argyi Folium by GC-MS/MS, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. **1181** (2021) 122904, doi: https://doi.org/10.1016/j.jchromb.2021.122904.
20. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonised Guideline: Validation of analytical procedures Q2(R2), 2022.
21. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonised Guideline: Bioanalytical method validation and study sample analysis M10, 2022.
22. *M. K. Woźniak, L. Banaszkiewicz, M. Wiergowski, E. Tomczak, M. Kata, B. Szpiech, J. Namieśnik, M. Biziuk,* Development and validation of a GC-MS/MS method for the determination of 11 amphetamines and 34 synthetic cathinones in whole blood, Forensic Toxicol. **38** (2020) 42–58, doi: https://doi.org/10.1007/s11419-019-00485-y.
23. *L. Rivier,* Criteria for the identification of compounds by liquid chromatography-mass spectrometry and liquid chromatography–multiple mass spectrometry in forensic toxicology and doping analysis, Anal. Chim. Acta **492** (2003) 69–82, doi: https://doi.org/10.1016/ S0003-2670(03)00889-4.
24. *R. P. Adams,* Identification of essential oil components by gas chromatography/mass spectrometry. 4th Edition, Allured Publ., Carol Stream, IL, 2007.

SUPPLEMENTARY MATERIAL

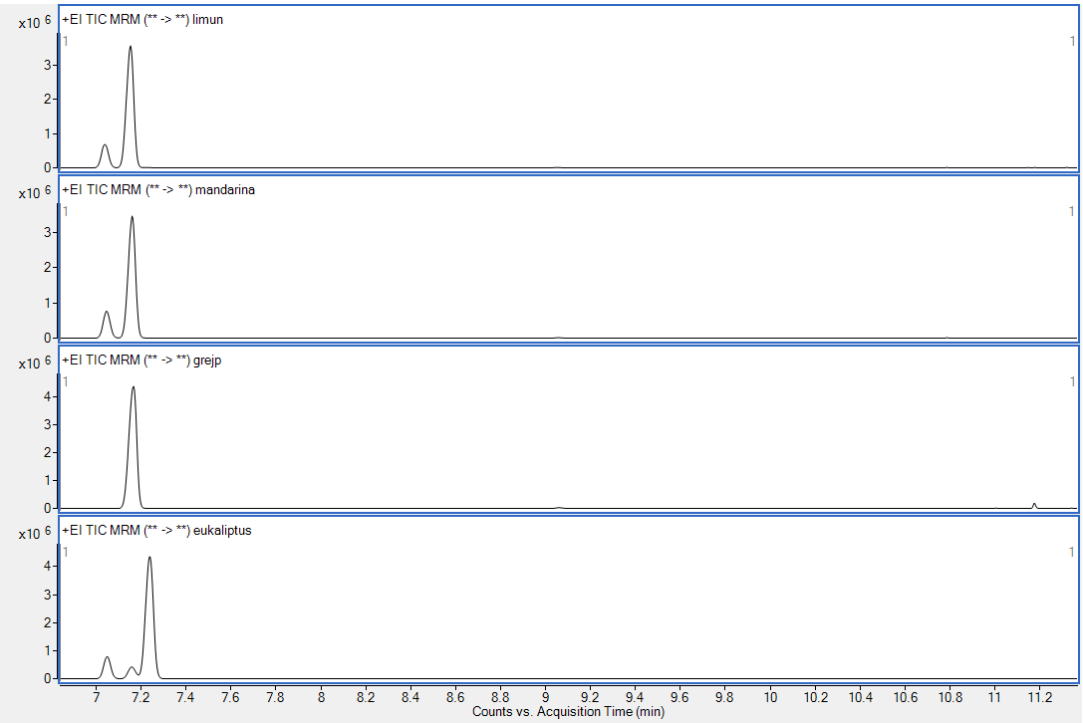

Fig. S1 – Chromatograms of lemon, tangerine, grapefruit and eucalyptus samples in top to bottom order
Slika S1 – Kromatogrami uzoraka limuna, mandarine, grejpa i eukaliptusa, redom od vrha do dna

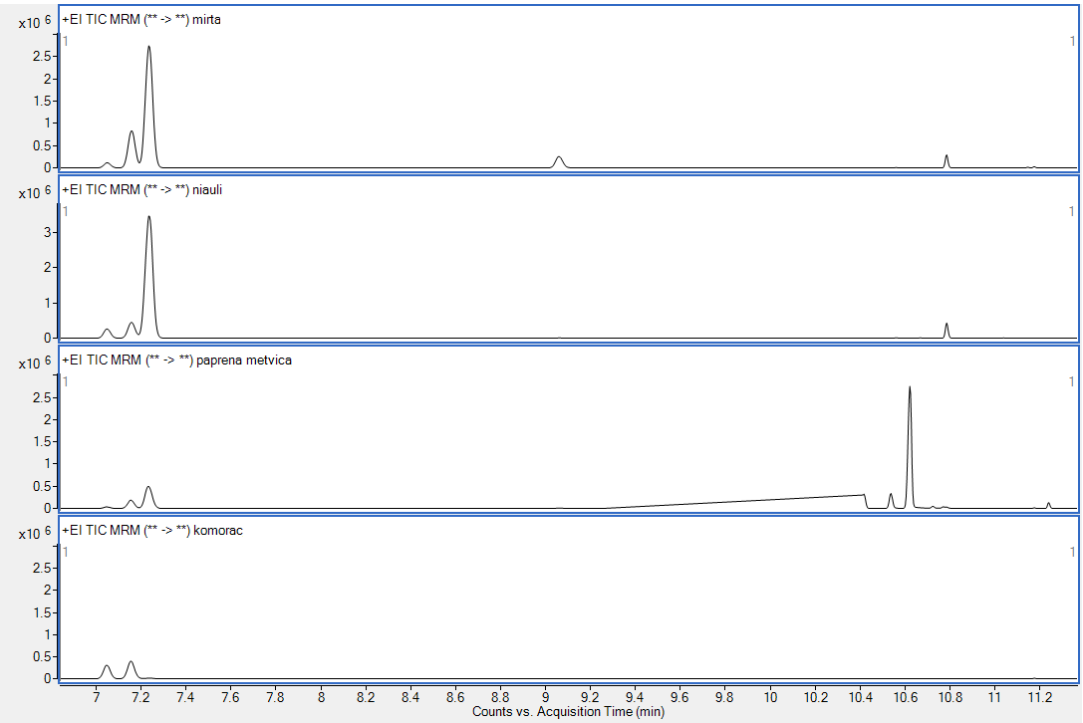

Fig. S2 – Chromatograms of myrtle, niaouli, peppermint and fennel samples in top to bottom order
Slika S2 – Kromatogrami uzoraka mirte, niaulija, paprene metvice i koromača, redom od vrha do dna

SAŽETAK

Razvoj i validacija GC-MS/MS metode za kvantitativno istodobno određivanje odabranih spojeva u eteričnim uljima

Ana Vučak, Glorija Golubić i *Franko Burčul**

Dobrobiti svakodnevne upotrebe eteričnih ulja povezuju se s poznatim antimikrobnim, protuupalnim i antioksidacijskim djelovanjem spojeva iz eteričnih ulja. S obzirom na to, ti se spojevi opsežno istražuju te postoji potreba za razvojem analitičkih metoda za njihovo jednostavno i brzo određivanje. U ovom je radu razvijena i vrednovana (validirana) metoda za kvantifikaciju *p*-cimena, limonena, eukaliptola, linalola, mentola i karvona u eteričnim uljima.

Tehnika primijenjena za odjeljivanje i određivanje tih spojeva je plinska kromatografija – tandemska spektrometrija masa (GC-MS/MS). Za pripremu otopina primijenjeni su standardi analita te heksan kao otapalo. Trostruki kvadrupolni spektrometar masa, u dMRM načinu rada, upotrijebljen je za praćenje specifičnih reakcija prijelaza pri njihovim optimalnim energijama sraza (kvantitativni i dva potvrdna prijelaza za svaki spoj). Plinska kromatografija optimizirana je promjenom temperaturnog gradijenta da bi se postiglo učinkovito odjeljivanje u kratkom vremenu (14 min).

Krivulje umjeravanja za svih šest spojeva pokazale su dobru linearnost ($R^2 \geq 0{,}998$), a koncentracijski raspon ovisi o analitu (0,10 – 10,00 μg ml^{-1}). Vrednovana je točnost (80,23 – 115,41 %), ponovljivost (≤ 12,03 %) i srednja preciznost (≤ 11,34 %) metode. Vrednovanje je provedeno prema ICH smjernicama, a ispitani parametri pokazali su se zadovoljavajućima. Time je osigurana prikladnost predložene metode za istodobno pojedinačno određivanje tih spojeva u eteričnim uljima. Metoda je uspješno primijenjena u analizi sljedećih eteričnih ulja: limun, mandarina, grejp, eukaliptus, mirta, niauli, paprena metvica i koromač.

Ključne riječi

Eterična ulja, GC-MS/MS, trostruki kvadrupol, MRM prijelaz, vrednovanje (validacija)

Sveučilište u Splitu, Kemijsko-tehnološki fakultet,
Ruđera Boškovića 35
21 000 Split, Hrvatska

Izvorni znanstveni rad
Prispjelo 27. lipnja 2024.
Prihvaćeno 17. kolovoza 2024.