Talanta 94 (2012) 140–145

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

The loss of essential oil components induced by the Purge Time in the Pressurized Liquid Extraction (PLE) procedure of *Cupressus sempervirens*

Andrzej L. Dawidowicz*, Natalia B. Czapczyńska, Dorota Wianowska

Department of Chromatographic Methods, Faculty of Chemistry, Maria Curie-Sklodowska University, pl. Maria Curie-Sklodowska 3, 20-031 Lublin, Poland

ARTICLE INFO

Article history:
Received 8 November 2011
Received in revised form 29 February 2012
Accepted 5 March 2012
Available online 10 March 2012

Keywords:
Purge Time
PLE
Volatile components
Sample preparation method
MSPD
SSDM

ABSTRACT

The influence of different Purge Times on the effectiveness of Pressurized Liquid Extraction (PLE) of volatile oil components from cypress plant matrix (*Cupressus sempervirens*) was investigated, applying solvents of diverse extraction efficiencies. The obtained results show the decrease of the mass yields of essential oil components as a result of increased Purge Time. The loss of extracted components depends on the extrahent type – the greatest mass yield loss occurred in the case of non-polar solvents, whereas the smallest was found in polar extracts. Comparisons of the PLE method with Sea Sand Disruption Method (SSDM), Matrix Solid-Phase Dispersion Method (MSPD) and Steam Distillation (SD) were performed to assess the method's accuracy. Independent of the solvent and Purge Time applied in the PLE process, the total mass yield was lower than the one obtained for simple, short and relatively cheap low-temperature matrix disruption procedures – MSPD and SSDM. Thus, in the case of volatile oils analysis, the application of these methods is advisable.

1. Introduction

The first step in the qualitative and quantitative analysis of plant constituents is the sample preparation procedure, the aim of which is to effectively and rapidly remove the analyte from its matrix. Solid liquid extraction is most frequently applied for this purpose. The choice of extraction technique is frequently decided upon consideration of operating costs, simplicity of operation, amount of organic solvent required and sample throughput. The traditional extraction methods (methods recommended in medicinal plant pharmacopeia, e.g. steam and water distillation, Soxhlet extraction, maceration, percolation, expression, cold fat extraction) have several shortcomings, including long extraction time and large consumption of solvents, cooling water and electric energy [1]. With the advent of laboratory automation and more and more wide-spread application of plant products in the pharmaceutical, medical, food and perfume industries, conventional extraction technologies are increasingly overlooked in routine analysis. Instrumental extraction methods requiring minimal sample handling are thus highly desirable [2]. Hence, several approaches are continuously being attempted in search of faster, cleaner and reliable analytical methodologies. As a response to such demands a number of techniques have been developed to meet the above criteria, for example, microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and Pressurized Liquid Extraction (PLE). The similarity between these techniques is the possibility of using elevated temperatures and pressures, which drastically improves the speed of the extraction process [3]. Raising the temperature increases the diffusion rates, the solubility of the analytes and their mass transfer, and decreases the viscosity and surface tension of the solvents. These changes improve the contact of the analytes with the solvent and enhance the extraction efficiency [4]. PLE has been shown to have significant advantages over competing techniques. For example, unlike MAE, in PLE no additional filtration step is required, since the matrix components that are not dissolved in the extraction solvent may be retained inside the sample extraction cell. This is very convenient for the purpose of automation and on-line coupling of extraction and separation techniques [5] which makes it more expensive than other assisted extraction methods (e.g. MAE). The principle of PLE is simple. The sample placed in the extraction cell is extracted with a solvent at a temperature ranging from ambient to 200 °C and at a relatively high pressure (from 4 to 20 MPa). In this approach, the selected solvent is pumped to fill the cell containing the sample, which is kept for a specified time at the selected pressure and temperature. Next, the extracted solvent is transferred to a collection vial. The sample and the connective tubings are then rinsed with a pre-selected volume of solvent. The inclusion of an additional nitrogen purge to guarantee the complete removal of the solvent from the PLE system is current practice. Together, these steps constitute a cycle and can be repeated several times if necessary. The total extraction time is

* Corresponding author. Tel.: +48 81 537 55 45; fax: +48 81 533 33 48.
E-mail address: dawid@poczta.umcs.lublin.pl (A.L. Dawidowicz).

0039-9140/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2012.03.008

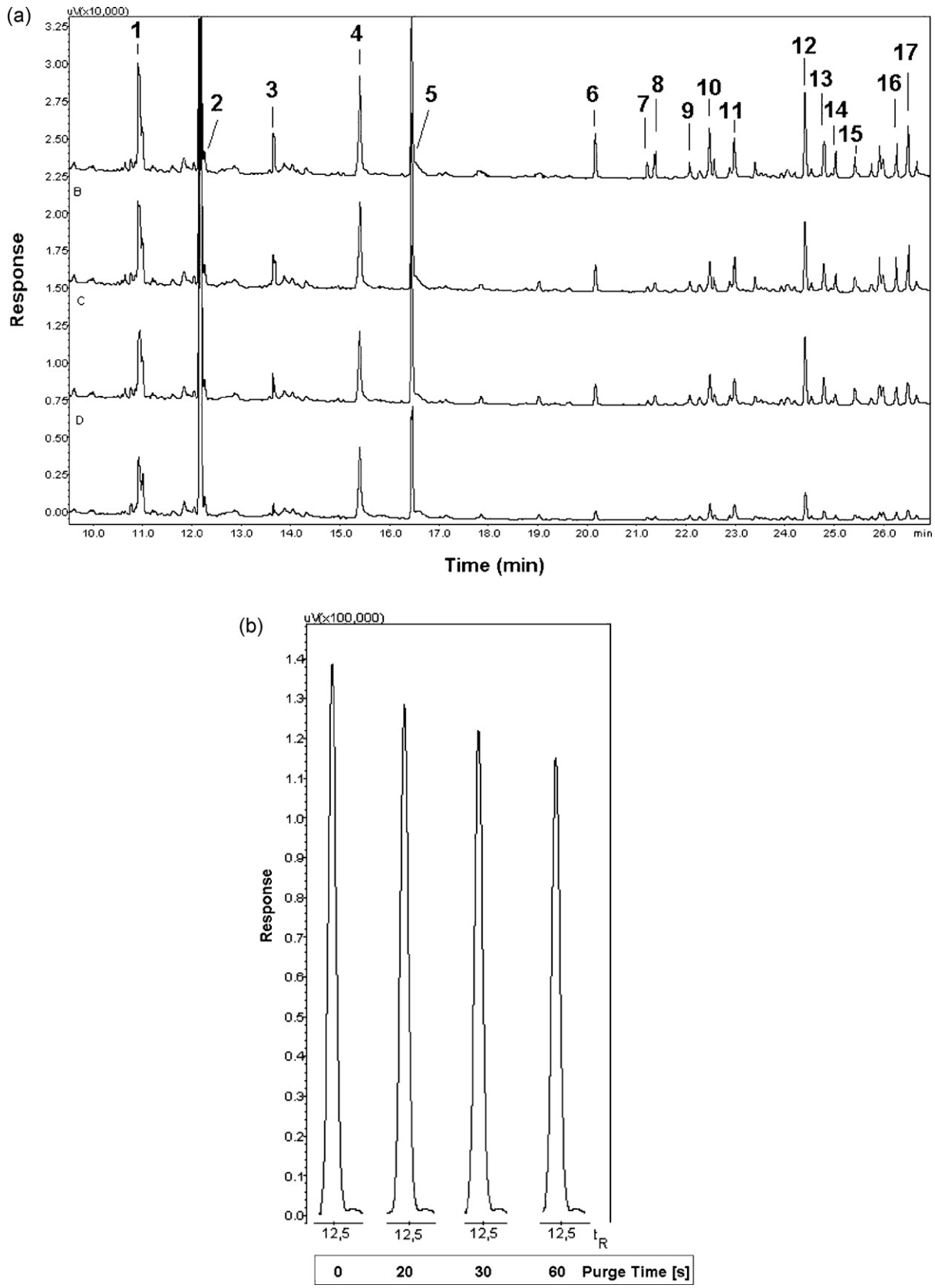

Fig. 1. (a) Exemplary GC-FID chromatograms of cypress extracts obtained by the means of PLE using ethyl acetate as an extraction solvent while applying different Purge Times 0 s (A), 20 s (B), 30 s (C) and 60 s (D). The peaks visible on chromatograms are: 1. β-Myrcene, 2. D-Limonene, 3. α-Terpinolene, 4. (−)-Terpinen-4-ol, 5. Standard, 6. Terpinyl acetate, 7. Carveol acetate, 8. Longifolene, 9. Thujopsene, 10. α-Humulene, 11. γ-Cadinene, 12. cis-Muurola-5-en-4-α-ol, 13. 1,2-epoxide-Humulene, 14. 1,10-si-epi-Cubenol, 15. 1-epi-Cubenol, 16. Hinesol, 17. epi-α-Cadinol. (b) The same as in (a) but for D-Limonene peak.

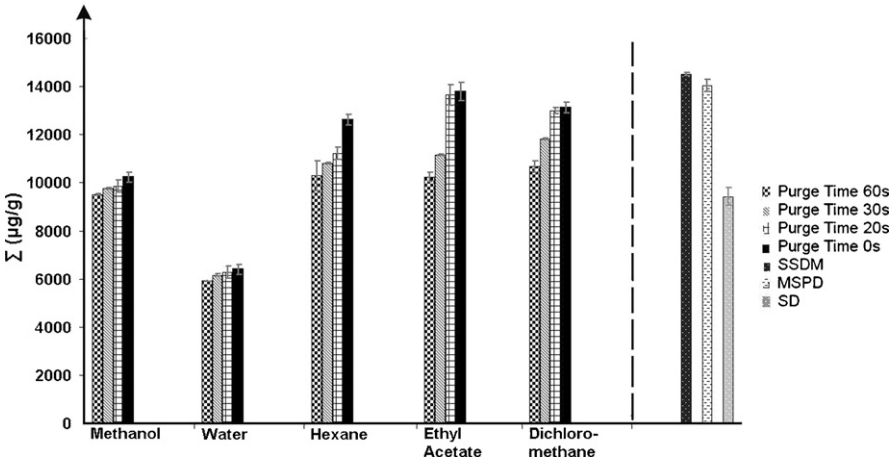

Fig. 2. Influence of Purge Time on the total yield of essential oil components estimated in cypress needles using PLE with different solvents – left side of the figure. The right side of the figure shows the total yield of essential oil components estimated by SSDM, MSPD and SD.

normally 15–45 min, although sometimes longer extraction time is necessary [6].

Evidence of the various applications of PLE in extraction and determination of various components from different matrices [3,7–17] have included the extraction of essential oil components from plant matrices such as mint (*Mentha piperita*), sage (*Salvia officinalis* L.), chamomile (*Chamomilla recutita* L.), marjoram (*Origanum majorana* L.), savory (*Satureja hortensis* L.) and oregano (*Origanum vulgare*) [18]. The last published results [19], however, showed that the yields of essential oils components estimated by both Matrix Solid Phase Dispersion (MSPD) and Sea Sand Disruption Method (SSDM) in coniferous trees needles are higher than those estimated by PLE. The higher volatility of coniferous essential oils raised the question of whether the lower efficacy of the PLE method in relation to MSPD and/or SSDM is accidentally due to the loss of components during the purge in PLE cycle.

Hence, the main objective of the current work was to evaluate whether PLE is adequate for volatile analytes – the most vulnerable to loss during the purge process. PLE was therefore tested applying different Purge Times. Cypress (*Cupressus sempervirens*) needles were chosen as the plant matrix, and solvents with various extraction forces (water, dichloromethane, ethyl acetate, methanol and hexane) were used in these experiments.

2. Experimental

2.1. Reagents and materials

Representative samples of cypress needles were collected in Lublin (Eastern Poland) in September 2011. Needles were stored at +4 °C to prevent degradation by light and temperature. Immediately before each extraction, plant material was ground and portions of identical weight were subjected to the sample preparation procedure.

Dodecane (Aldrich, Gilingham, UK) dissolved in n-octane (Merk, Germany) (51.2 mg dodecane in 50 mL of n-octane and/or 75.6 mg dodecane in 50 mL of n-octane) was used as the internal standard.

1,4-Dioxane, ethyl acetate, methanol, dichloromethane and hexane were purchased by POCH (Gliwice, Poland).

The C_{18} sorbent (Sepra C_{18}, 50 μm, 65 Å) for MSPD was supplied by Phenomenex (Torrance, CA, USA).

The sand, Brazilian quartz, was fractionated, leached with 1 M hydrochloric acid, washed out with distilled water to neutrality and dried. 80–100 μm fraction was applied for the SSDM process and as a filling material of PLE extraction cells.

2.2. Pressurized liquid extraction

PLE was performed with a Dionex ASE 200 instrument (Dionex, Sunnyvale, CA, USA). Samples of needles (0.5 g) were accurately weighed and mixed together with sand. They were then placed into a 22 mL stainless steel extraction cell with a cellulose filter at the bottom ends. The sample cells were then closed to finger tightness and placed into the carousel of the ASE 200 system. Ethyl acetate or methanol or dichloromethane or hexane and/or water were used as the extraction solvents. Extractions were carried out at 100 °C and at operating pressure of 40 bar. Each extraction lasted 10 min. After the extraction process, the extraction cell content was flushed using the same solvent in the amount equal to 60% of the extraction cell volume, and purged for 60 or 30 or 20 or 0 s by applying pressurized nitrogen (at 150 psi). All extraction procedures were repeated 3 times. An appropriate amount of internal standard was added to the extract and subjected to GC analysis. The quantity of each component of essential oil was calculated as relative concentration (peak area percentage) and as the amount (μg/g), recalculated according to the internal standard.

2.2.1. Solid phase extraction of aqueous and methanolic extracts

Before GC analysis, essential oil components were re-extracted from water and methanol extracts using SPE procedure. 6 mL of water extract was mixed with 4 mL of methanol. 2 mL of the obtained solution were loaded onto a 0.5 g Sepra C_{18}-E SPE cartridge previously washed with 5 mL of methanol and 5 mL of 40% methanol (aq), and dried completely by means of a vacuum (ca. 8 min). Essential oil compounds were eluted with hexane–ethyl acetate mixture (9:1, v/v) to 5 mL calibrated flasks. An appropriate amount of internal standard was added to the extract and subjected to GC analysis. In the case of methanolic extracts of essential oils, 4 mL of methanolic extract was mixed with 6 mL of water and 2 mL of the obtained solution were loaded onto SPE cartridge. The next steps of the SPE procedure were the same as above. The SPE conditions of essential oil components isolation from aqueous and methanolic extracts agree with those elaborated for the SPE fractionation of essential oil components [20].

2.3. Steam distillation

Steam distillation was performed according to the European Pharmacopeia recommendations using a Clevenger-type apparatus. 10 g of fresh needles and 500 mL of water were subjected to a steam distillation process which lasted 3 h. An appropriate amount

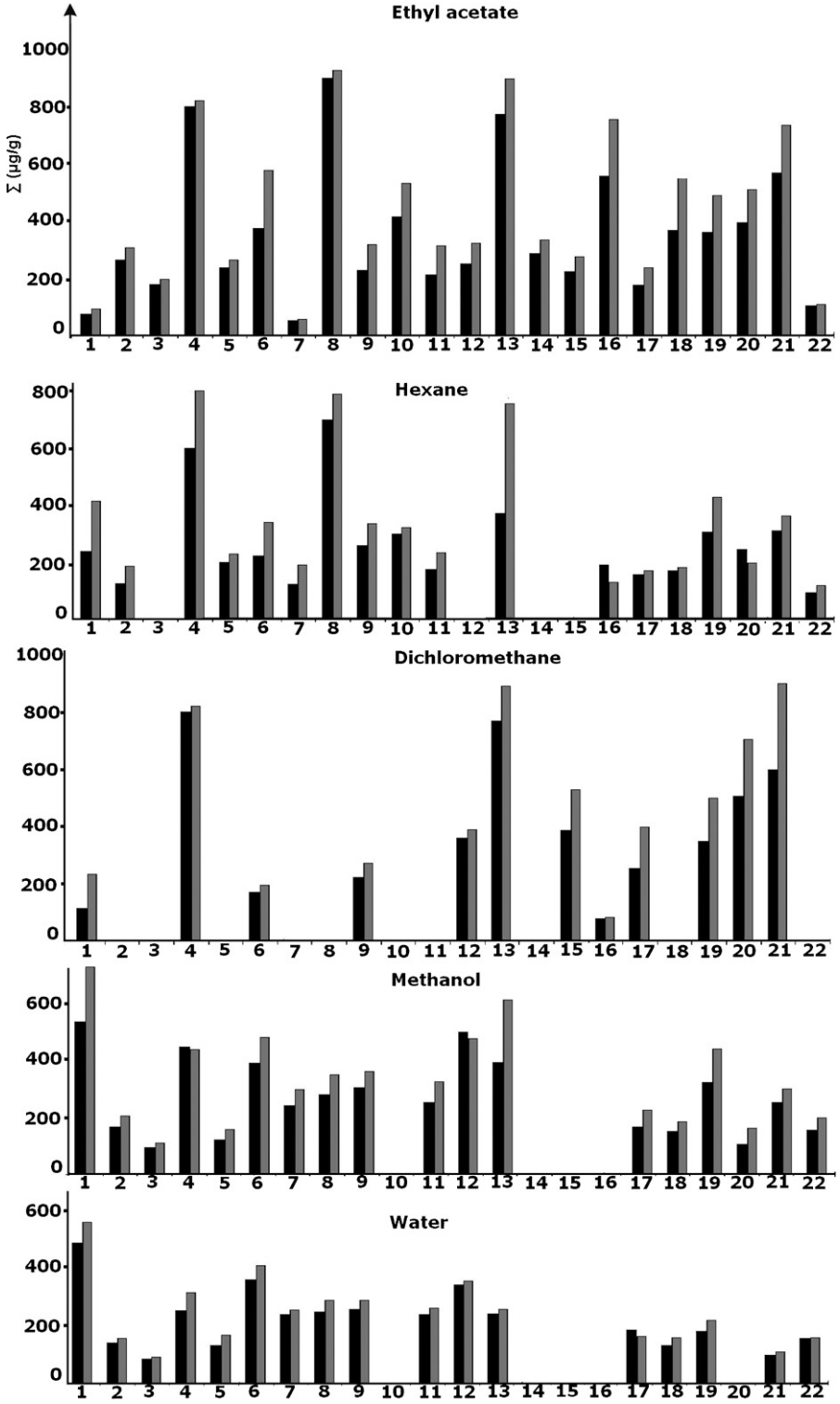

Fig. 3. The various yields of individual of essential oil components estimated by PLE in cypress needles at different Purge Times (0 and 60 s) and applying different solvents. Essential oil components in order: 1. α-Pinene, 2. (+)-Sabinene, 3. 1-Octen-3-ol, 4. β-Myrcene, 5. α-Terpinene, 6. α-Terpinolene, 7. 1,3,8-p-Menthatriene, 8. (−)-Terpinen-4-ol, 9. δ-Elemene, 10. Terpinyl acetate, 11. Longifolene, 12. Thujopsene, 13. α-Humulene, 14. γ-Cadinene, 15. cis-Muurola-5-en-4-β-ol, 16. cis-muurola-5-en-4-α-ol, 17. 1,2-epoxide-Humulene, 18. 1,10-si-epi-Cubenol, 19. 1-epi-Cubenol, 20. Hinesol, 21. epi-α-Cadinol, 22. α-Cadinol.

of the internal standard solution was added prior to the process. The procedure was repeated 3 times, each time with a fresh portion of needles. The obtained essential oil samples were subjected to GC analysis. The quantity of each component of essential oil was calculated as relative concentration (peak area percentage) and as the amount (μg/g), recalculated according to the internal standard.

2.4. Sea Sand Disruption Method

A sample of cut needles (0.2 g) was placed in a glass mortar with the sand (4.8 g) and 3 mL of 1,4-dioxan [19]. The materials were mixed for 10 min, using a glass pestle to obtain a homogenous material suitable for column packing. The blend was then quantitatively

transformed into a 5 mL syringe barrel containing a filter paper at the bottom. Plant components were then eluted to 10 mL calibrated flask using ethyl acetate. All analyzed samples were prepared in triplicate. An appropriate amount of internal standard was added to the extracts and subjected to GC analysis. The quantity of each component of essential oil was calculated as relative concentration (peak area percentage) and as the amount (μg/g), recalculated according to the internal standard.

2.5. Matrix solid phase dispersion

A sample of cut needles (0.2 g) was placed in a glass mortar with the Sepra C_{18}-E sorbent (0.8 g) (the most encountered mass ratio 1:4) and 1 mL of 1,4-dioxan. The materials were mixed for 10 min, using a glass pestle to obtain a homogenous material suitable for column packing. The blend was then quantitatively transformed into a 5 mL syringe barrel containing a filter paper at the bottom. Plant components were then eluted to 10 mL calibrated flask using ethyl acetate [21]. All analyzed samples were prepared in triplicate. The quantity of each component of essential oil was calculated as relative concentration (peak area percentage) and as the amount (μg/g), recalculated according to the internal standard.

2.6. Chromatographic analysis

Qualitative analysis of the examined extracts was performed on GC/MS from Shimadzu (QP2010 System, Kyoto, Japan). A ZB5-MS fused silica capillary column (30 m length × 0.25 mm ID, film thickness of 0.25 μm) (Phenomenex, USA) was directly coupled to the mass spectrometer. One microliter samples were injected using the 10C-20i type autosampler. Helium with the flow rate 1.0 mL/min was used as carrier gas. During the chromatographic separation the oven temperature increased from 50 °C to 310 °C at 6°/min ramp rate. In the mass spectrometer unit the following conditions were used: ion source temperature of 220 °C, ionization voltage of 70 eV. The mass spectra were measured in the range 35–360 amu. Qualitative analysis was carried out comparing the obtained MS spectra with the NIST'05 library spectra. The presence of a given component was additionally confirmed by the published and our own temperature retention indexes. Authentic data reported in literature was also referred to.

Quantification of extracts was performed using a gas chromatogram from Shimadzu, model GC-2010, equipped with Flame Ionization Detectors (FID) under the following conditions: the ZB5-MS fused-silica capillary column (30 m length × 0.25 mm ID, film thickness of 0.25 μm), the sample volume injected: 1 μm. The temperature program during GC-FID separation was the same as for GC–MS.

Quantification of essential oil components was made by comparing the areas of their chromatographic peaks versus the dodecane (internal standard), a known amount of which was added to the examined extracts before GC measurements.

3. Results and discussion

Fig. 1 presents four exemplary chromatograms of cypress essential oil components extracted by the means of PLE using ethyl acetate as an extraction solvent. Four extractions were made in exactly the same conditions including the extraction time, temperature and pressure. For clarity of Fig. 1, the names of the individual essential oil components were omitted and listed in the legend. The Purge Time was the only factor differentiating these extractions. The presented results indicate an increase in the mass yields of essential oil components with the Purge Time decrease – the highest are found when there is no Purge Time (0 s), whereas the lowest occur as a result of applying 60 s Purge Time. The obtained results

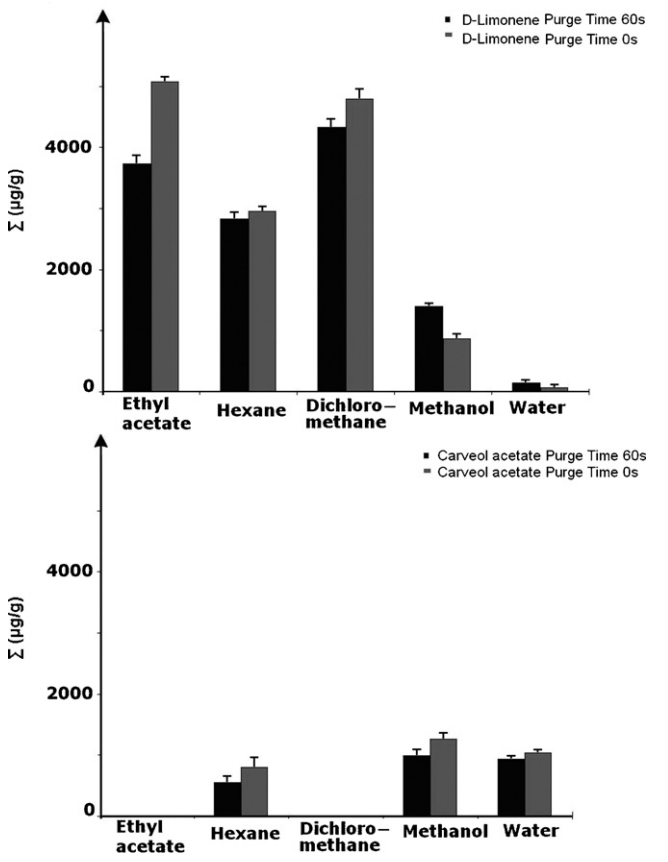

Fig. 4. The yields of the main essential oil component – D-Limonene, and the product of its oxidation – Carveol acetate, estimated by PLE in cypress needles at different Purge Times (0 and 60 s) and applying different solvents.

unequivocally prove that Purge Time has a very significant impact on the total mass yield's estimation while examining volatile components in solid samples using PLE as an isolation method.

This phenomenon is the most pronounced in Fig. 2, which shows the influence of Purge Time on the total quantity of essential oil components depending on extraction solvent applied. Indeed, it can be seen that the total amount of essential oils components yield is strongly dependent upon the solvent chosen. The majority of essential oil components are of lipophilic character, so are better soluble in less polar organic solvents like hexane, dichloromethane or ethyl acetate and hence, the greatest yields of total essential oil components obtained by applying these solvents are apparent. The extraction efficiency of methanol and water is therefore significantly lower. However, the influence of Purge Time on the total yield of essential oil components in these polar solvents is less pronounced than in more lipophilic ones. This observation suggests that the loss of essential oil components is due to not only the evaporation of essential oil components during the purge with the nitrogen stream but also can be connected to the evaporation process of solvent itself from plant extract in the gas stream. It can be assumed that essential oil components undergo the co-evaporation process together with solvent molecules, in a manner similar to co-distillation.

Fig. 3 presents the influence of varying Purge Times (60 s, 0 s) on the estimation of individual essential oil component quantities. The main oil's component; D-Limonene, as well as the product of its oxidation, Carveol acetate, have been presented separately in Fig. 4 so as to display more clearly the changes that occurred. In the interests of clarity for Fig. 3, the names of the individual essential oil components were omitted and listed in the legend. In order

to simplify Fig. 4 it was decided to limit the number of volatile components considered for the cypress essential oil. Only these components which had a concentration above 1% of total essential oil components yield were taken into consideration. As illustrated in Fig. 3, Purge Time leads to the loss of almost every component regardless of solvent applied. The data from Fig. 3 extends the previous conclusion concerning the influence of the extrahent type on the difference in the mass yields of essential oil components estimated by PLE with different Purge Times. However, in this case and in relation to the individual components – the smallest differences in the mass yield of essential oil components occur in the case of more polar solvents (methanol and water), whereas the biggest differences are found for less polar solvents (ethyl acetate, hexane and dichloromethane). Due to the different extraction efficiencies and characters of the solvents applied in this experiment, some of the essential oil components were extracted in trace amounts (empty spaces in Fig. 3). The significance of this fact, however, has been overlooked as the influence of the Purge Time on essential oil components quantities, not the differences between the solvent extraction efficiencies, is the subject of this paper.

Similar conclusions can be driven from Fig. 4 in which the changes in the mass yield of the main essential oil component, D-Limonene, and the product of its oxidation, Carveol acetate, are presented. In both cases, the application of longer Purge Time has a negative impact on their extraction. Its worth mentioning that the presence of Carveol acetate is specific for methanol, water and surprisingly for hexane. The minute quantities of Carveol acetate are found in the ethyl acetate and dichloromethane extracts.

The RSD range for Figs. 3 and 4 was from 0.2% to 6.5%.

4. Conclusions

The results presented in the paper show that the quantities of essential oil components estimated by the means of PLE depend on Purge Times applied as part of the PLE process – the longer Purge Time, the greater the loss of volatile components within essential oil. The loss of volatile components in the PLE process is also affected by the type of the extrahent used. Examining essential oil derived from cypress needles, it was verified that the greatest mass yield losses occur in the case of non-polar solvents, whereas the smallest losses were found in polar extracts. Regardless of the solvent and Purge Time applied in the PLE process, the total mass yield in PLE is lower than the one obtained for simple, short and relatively cheap low-temperature matrix disruption procedures – MSPD and SSDM. Thus, in the case of volatile oils analysis, the application of these methods is advisable. Therefore, a reasonable approach to tackle this issue could be to make large randomized controlled trials for the greater number of plant matrices containing the volatile components of diverse chemical character.

References

[1] A.L. Dawidowicz, D. Wianowska, J. Pharm. Biomed. Anal. 37 (5) (2005) 1155–1159.
[2] B. Kaufmann, P. Christien, J. Phytochem. Anal. 13 (2002) 105–113.
[3] B. Benthin, H. Danz, M. Hamburger, J. Chromatogr. A 837 (1999) 211–219.
[4] L. Ramosa, E.M. Kristensonb, U.A.Th. Brinkmanb, J. Chromatogr. A 975 (1) (2002) 3–29.
[5] R. Carabias-Martínez, E. Rodríguez-Gonzalo, P. Revilla-Ruiz, J. Hernández-Méndez, J. Chromatogr. A 1089 (1–2) (2005) 1–17.
[6] A. Nieto, F. Borrull, E. Pocurull, R.M. Marc, Trends Anal. Chem. 29 (7) (2010) 752–764.
[7] T. Mroczek, J. Mazurek, Anal. Chim. Acta 633 (2009) 188–196.
[8] T.L. Miron, M. Plaza, G. Bahrim, E. Ibáñez, M. Herrero, J. Chromatogr. A 1218 (2011) 4918–4927.
[9] N. Barco-Bonilla, J.L. Martínez Vidal, A. Garrido Frenich, R. Romero-González, Talanta 78 (2009) 156–164.
[10] G. Zgórka, Talanta 79 (2009) 46–53.
[11] R. Assis Jacques, C. Dariva, J.V. de Oliveira, E. Bastos Caramão, Anal. Chim. Acta 625 (2008) 70–76.
[12] L. Jaime, I. Rodríguez-Meizoso, A. Cifuentes, S. Santoyo, S. Suarez, E. Ibáñez, F.J. Señorans, LWT 43 (2010) 105–112.
[13] J. Hua, Z. Guo, M. Glasius, K. Kristensen, L. Xiao, X. Xu, J. Chromatogr. A 1218 (2011) 5765–5773.
[14] I. Borrás Linares, D. Arráez-Román, M. Herrero, E. Ibáñez, A. Segura-Carretero, A. Fernández-Gutiérrez, J. Chromatogr. A 1218 (2011) 7682–7690.
[15] W. Li, Z. Wang, L. Chen, J. Zhang, L. Han, J. Hou, Y. Zheng, J. Sep. Sci. 33 (2010) 2881–2887.
[16] M. Herrero, M. Plaza, A. Cifuentes, E. Ibáñez, J. Chromatogr. A 1217 (2010) 2512–2520.
[17] E. Pérez-Torrado, J. Blesa, J.C. Moltó, G. Font, Food Control 21 (2010) 399–402.
[18] A.L. Dawidowicz, E. Rado, D. Wianowska, M. Mardarowicz, J. Gawdzik, Talanta 76 (2008) 878–884.
[19] A.L. Dawidowicz, N.B. Czapczyńska, Chem. Biodivers. 8 (2011) 2045–2056.
[20] A.L. Dawidowicz, M.P. Dybowski, J. Sep. Sci. 33 (2010) 3213–3220.
[21] A.L. Dawidowicz, E. Rado, J. Pharm. Biomed. Anal. 52 (2010) 79–85.