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Assay of terpene alcohols in pharmacopoeial essential oils by micellar electrokinetic capillary chromatography (MEKC)

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Micellar electrokinetic capillary chromatography (MEKC) was used to separate and determine terpene alcohols of wide occurrence in herbal extracts and essential oils, namely eugenol, linalool, geraniol, citronellol and thymol. In the present paper sodium dodecyl sulfate (SDS) has been used as a micelle-forming additive to the CZE background electrolytes. Effects of SDS concentration, buffer type, its pH and concentration, addition of organic solvents on the migration times and separation efficiency were investigated. The optimal electrolyte system consisted of 20 mM TAPSO and 30 mM SDS in aqueous 10% (v/v) acetonitrile of pH 7.5 (adjusted by the addition of TRIS). The separation capillary was a fused silica tube (50 μm I. D., total length 75 cm, 42 cm effective length) maintained at 25 °C. The separations were performed at the applied voltage of 20 kV. Samples were injected hydrodynamically at a pressure of 50 mbar for 6 s. Detection was carried out at 200 nm. The calibration curves were rectilinear for 50–200 mg l^{-1} (for eugenol, thymol and geraniol) and 100–400 mg l^{-1} (for linalool and citronellol). The limits of detection varied between 5 mg l^{-1} (for thymol) and 16 mg l^{-1} (for linalool). The devised MEKC method was employed for the determination of the cited terpene alcohols as major quality-affecting constituents in commercial pharmacopoeial essential oils such as *Geranii etheroleum*, *Caryophylli floris etheroleum*, *Lavandulae etheroleum* and *Thymi etheroleum*. The results agreed well with those of a reference gas chromatographic method.

1. Introduction

Separation by capillary electrophoresis (CE) is based on differences in the electrophoretic mobilities of solutes. Although CE is inherently a highly efficient separation technique, it is necessary to evaluate experimental conditions carefully to achieve maximum separation selectivity. For weakly acidic or basic solutes, buffer pH selection is probably the most important among the experimental variables. The effective electrophoretic mobilities can be modified by optimizing buffer pH to maximize the mobility differences and achieve highest resolution between closely migrating solute pairs. For solutes with similar pK_a values pH control alone cannot improve resolution.

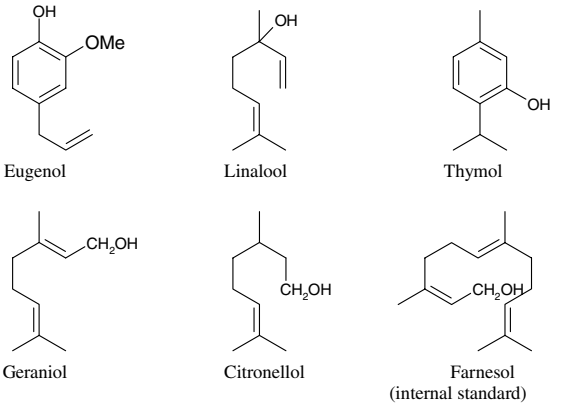
In 1984 Terabe and co-workers introduced a modified version of capillary electrophoresis, the micellar electrokinetic chromatography (MEKC) (Terabe et al. 1984; Otsuka and Terabe 1996). Through the inventive use of micellized surfactants as part of the electrolyte medium, the scope of the electrophoretic technique to separate and analyze neutral compounds was extended. In terms of separation principles, MEKC is based on both different electrophoretic mobilities and different partition coefficients between two liquid phases, and has to be regarded therefore, as a combination of chromatography and electrophoresis. Both the primary (mobile) and secondary (micellar) phases can be changed to allow changes in retention and to provide a better separation (Nishi and Terabe 1990; Holland and Sepaniak 1993). Generally in MEKC anionic surfactans forming micelles, especially sodium dodecyl sulfate (SDS), are used most frequently. In comparison with neutral surfactans, anionic surfactans have a greater potential for separation of neutral solutes because the negatively charged micelles migrate more slowly than the electro-osmotic flow (EOF) and do not adsorb onto the negatively charged wall of a fused silica capillary (Li and Locke 1996). Even though MEKC is generally not quite as efficient a technique as normal CE, MEKC extends many of the advantages of CE to the separation of neutral molecules.

The most frequently used method in the analysis of volatile neutral terpenoids is gas chromatography (GC) (Píry et al. 1995; Venskutonis 1997; Porter et al. 1999; Rojas et al. 1999; Kim et al. 2000; Falqué et al. 2001; Rapparini et al. 2001; Paúl et al. 2002; Rohloff 2002) followed by liquid chromatography (HPLC) (Ochocka et al. 1992; Ney et al. 1996; Verzera et al. 2000; Ehlers et al. 2001). As an alternative to the classical techniques, CE would show the following advantages compared to GC and HPLC: shorter time of analysis, limited use of organic solvents and consequently minimum production of toxic wastes.

Our literature search revealed that only a few articles reported analysis of neutral terpenes by CE techniques. The micellar electrokinetic chromatography was used recently

for the determination of monoterpenes such as camphene, α-pinene, β-pinene, α-terpinene, terpinolene and p-cymene in synthetic samples (Rodrigues et al. 2001). Addition of cyclodextrins to the buffer solution containing 50 mM SDS, 10 mM NaH_2PO_4, and 6 mM $Na_2B_4O_7$ resulted in improved separation of these monoterpenes; the best separation was achieved with γ-cyclodextrin. Cold-pressed orange oil was analysed for carvon, myrcene, limonene, citral and linalool by MEKC (Ogawa et al. 2002) with BGE containing 50 mM SDS in 20 mM phosphate buffer of pH 8.1. The MEKC method developed was compared with capillary liquid chromatography. Rodrigues and coworkers (Rodrigues et al. 2002) used MEKC with BGE consisting of 10 mM NaH_2PO_4, 6 mM $Na_2B_4O_7$, 50 mM SDS, 7 mM γ-cyclodextrin and 10% acetonitrile of pH 8.0 for the analysis of majoram essential oils for five terpene hydrocarbons and three terpene alcohols (linalool, α-terpineol and terpinen-4-ol).

About 17 essential oils are described in the European Pharmacopoeia 2002 (Ph. Eur. 2002). For their quality control the methods of gas chromatography are used most frequently. These methods are rather time-consuming (for example the retention time is about 47 min for linalool in the GC pharmacopoeial analysis of *Lavandulae etheroleum*. and approximately 42 min for eugenol in the analysis of *Caryophylli floris etheroleum*). MEKC could be a rapid alternative method for routine determination of terpenes in pharmacopoeial essential oils.



The aim of this investigation was to develop a rapid electrophoretic method for the separation and quantitative assay of monoterpene alcohols such as eugenol, linalool, geraniol, citronellol and thymol in pharmacopoeial essential oils. These compounds are also of cosmetologic importance since they are present in most personal care products (soap and toiletries) and exquisite perfumes. Moreover, they find large application in the food and drink industry as flavour enhancers. In the present paper sodium dodecyl sulfate (SDS) has been selected as a micelle-forming additive to the CZE background electrolytes. Effects of SDS concentration, buffer type, its pH and concentration, addition of organic solvents, voltage and temperature on the migration times and separation efficiency were investigated. The optimised MEKC method was used for the determination of the specified terpene alcohols as major quality-affecting constituents in commercial pharmacopoeial essential oils namely *Geranii etheroleum*, *Caryophylli floris etheroleum*, *Lavandulae etheroleum* and *Thymi etheroleum*. To our best knowledge this was the first attempt of quantitative analysis of pharmacopoeial essential oils for the terpene alcohols by a capillary electro-migration method. Validation of the proposed MEKC method involved evaluation of the accuracy of results by a reference gas chromatographic method (Kim et al. 2000).

2. Investigations, results and discussion

2.1. Chemical variables

Preliminary experiments indicated that the concentration of SDS and the kind and amount of organic solvent added to the buffer solution have the main influence on the separation efficiency and migration times of compounds analyzed. Several types of buffer solutions with different pKa values were examined (MES, HEPPSO, TAPSO). Regular and most symmetric peak shapes were attained with TAPSO (pKa 7.6) of pH 7.5 (adjusted with TRIS) containing SDS as the BGE; in this electrolyte system also the highest analyte peaks were observed (compared to BGEs based on MES (pKa 6.1) or HEPPSO (pKa 6.8). The effect of concentration of TAPSO (10 to 30 mM, pH 7.5) in the BGE with fixed concentration of SDS (30 mM) was examined. With increasing concentration of TAPSO the migration times of analytes increased considerably. Optimum concentration of TAPSO was 20 mM (the migration time of citronellol as the "slowest" analyte did not exceed 10 min). In further experiments the BGE with fixed concentration of TAPSO (20 mM; pH 7.5) and varying concentration of SDS (10–40 mM) were examined. The optimum concentration of SDS was 30 mM because it offers a compromise between the analysis time and resolution. For 10 mM SDS the compounds were not separated and at 40 mM SDS the migration times increased considerably. At 30 mM SDS the separation efficiency was highest but for the peaks of linalool, thymol, geraniol and citronellol only partial separation was achieved. Addition of 10% of methanol to the BGE lead to better separation of the analytes except of thymol and linalool that migrated together. An increased concentration of methanol in the BGE did not improve the separation. The best resolution was achieved after the addition of 10% of acetonitrile to the BGE. The migration times decreased and the peaks of compounds were separated down to the baseline. Thus the optimum BGE for the MEKC separation was 20 mM TAPSO in aqueous 10% (v/v) acetonitrile of pH 7.5 containing 30 mM SDS. A typical electrophoreogram of a model mixture of the monoterpene alcohols including farnesol as potential internal standard obtained under optimal separation conditions is shown in Fig. 1.

2.2. Quantitative analysis – calibration curves and figures of merit

Calibration curves relating the analyte peak area/internal standard peak area ratio to concentration of the analyte were measured with five calibration solutions containing 50–200 mg l^{-1} of eugenol, thymol and geraniol, 100–400 mg l^{-1} of linalool and citronellol and 100 mg l^{-1} of farnesol as internal standard. Each calibration point was measured in five replicates with the optimum BGE under the separation conditions given in the Experimental section. At the same time the migration times of the analytes were determined and the repeatability of the peak areas at concentration level of 100 mg l^{-1} of eugenol, thymol and geraniol and 200 mg l^{-1} of linalool and citronellol was evaluated. The linear regression parameters of calibration curves, migration times determined and corresponding RSD values are summarized in Table 1. It can be seen that

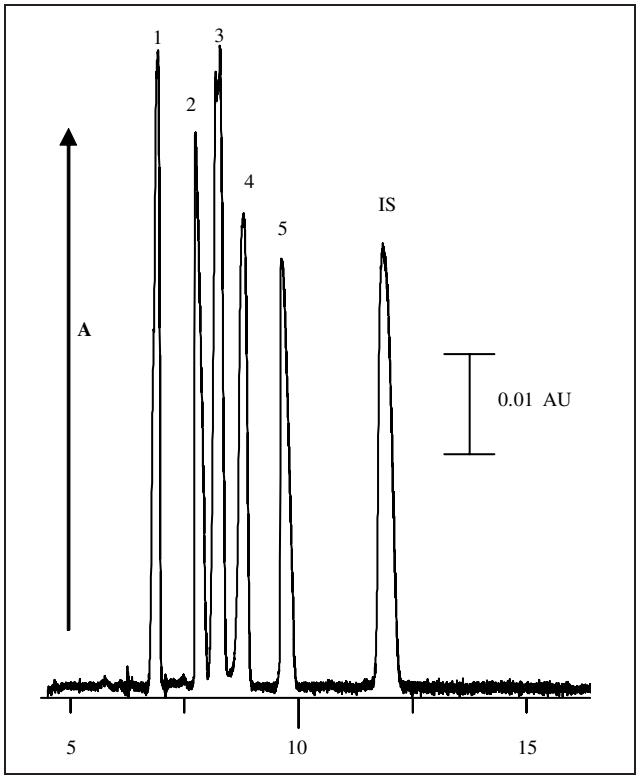


Fig. 1: Separation of a model mixture of terpene alcohols by MEKC; injected sample contains 100 mg/l of eugenol (1), 200 mg/l of linalool (2), 100 mg/l of thymol (3), 100 mg/l of geraniol (4), 200 mg/l of citronellol (5) and 100 mg/l of farnesol (IS). For separation conditions see Experimental

the repeatability is fairly good since the RSD values of migration times and peak areas do not exceed 2.1%. The limit of detection (LOD) values were calculated from the linear regression data as 3.3 SD_i/slope where SD_i stands for the standard deviation of intercept; similarly the limit of quantification (LOQ) values were estimated as 10 SD_i/slope (Rodrigues et al. 2001, 2002). The limits of quantification varied between 14 mg l^{-1} (for thymol) and 48 mg l^{-1} (for linalool). These LOQ values are fully sufficient for the analysis of real essential oil samples since the content of individual major monoterpene alcohols in pharmacopoeial essential oils typically exceeds 10%.

2.3. Determination of the monoterpene alcohols in commercial pharmacopoeial essential oils

The MEKC method developed has been applied to the determination of major monoterpene alcohols in commercial pharmacopoeial essential oils *Geranii etheroleum* (linalool, geraniol, citonellol), *Caryophylli floris etheroleum* (eugenol), *Lavandulae etheroleum* (linalool) and *Thymi etheroleum* (thymol). The electrophoreograms of these real samples are shown in Fig. 2. The peaks of these five analytes were identified by spiking the test solutions successively with the monoterpene standard solutions that resulted in the increase of the heights of the appropriate peaks. Quantification was carried out using calibration curves of the individual analytes and the content of the monoterpenes was calculated as their percentage in the essential oils analysed. The accuracy of the results was evaluated by the added-found (recovery) experiments; to estimate the recovery the essential oils, initially analysed by MEKC, were treated with the analyte standards to increase their concentration by 100 mg l^{-1} and thereafter they were subjected to additional MEKC assay. The recovery values (~96% to 99%) indicate that the proposed method is sufficiently accurate. The accuracy of the results of the MEKC assay of original essential oil samples was also tested by comparison with the results obtained by a reference GC method (Kim et al. 2000) with use of the Student's t-test (Eckshlager et al. 1980). The results of the MEKC and GC analyses of real samples are reported in Table 2. The Student's t-test did not reveal any statistically significant difference between the results obtained by the proposed MEKC and reference GC assay.

Apparently the proposed MEKC method is suitable for the assay of monoterpene alcohols in commercial pharmacopoeial essential oils. From six terpene alcohols determined by our proposed MEKC method only linalool was determined earlier by MEKC in orange oil (Ogawa et al. 2002) or in *Majorana hortensis* essential oil samples (Rodrigues et al. 2002). Compared to gas chromatography (GC) which is the officinal method for the quality control of pharmacopoeial essential oils the MEKC separation is substantially less time consuming (the separation of real samples takes about 10 min in contrast to > 30 min for GC). The MEKC method is sufficiently sensitive, reproducible and the results do not show statistical difference from those of GC.

Table 1: Migration times (t_m), CE calibration parameters and figures of merit for monoterpene alcohols determined by the proposed CE method

Analyte	t_m (min) (RSD; n = 5)	y = kx + q*	r	Area RSD** (n = 5)	LOD (mg l^{-1})	LOQ (mg l^{-1})
Eugenol		k = 0.418 ± 0.005				
	7.23 (1.7)	q = 1.347 ± 0.848	0.9997	2.1	20	67
Linalool		k = 0.132 ± 0.003				
	7.68 (0.9)	q = −1.154 ± 0.638	0.9994	0.4	16	48
Thymol		k = 0.383 ± 0.004				
	7.95 (2.1)	q = −0.031 ± 0.532	0.9998	1.9	5	14
Geraniol		k = 0.248 ± 0.003				
	8.31 (1.6)	q = −0.348 ± 0.516	0.9996	2.0	7	21
Citronellol		k = 0.145 ± 0.002				
	9.89 (0.7)	q = −2.385 ±0.627	0.9995	1.5	14	43

x: analyte concentration (mg · l^{-1}); y: peak area ratio (analyte/internal standard)
k = slope; q = intercept; r = correlation coefficient; n = number of experiments; RSD = relative standard deviation of the peak area and electrophoretic migration time values t_m; LOD = limit of detection; LOQ = limit of quantification. Concentration, LOD and LOQ are expressed in mg · l^{-1}
* five calibration points; each calibration point was measured in five replicates
** calculated from the measured peak areas for calibration solutions containing 100 mg l^{-1} of eugenol, thymol and geraniol and 200 mg · l^{-1} of linalool and citronellol (five measurements)

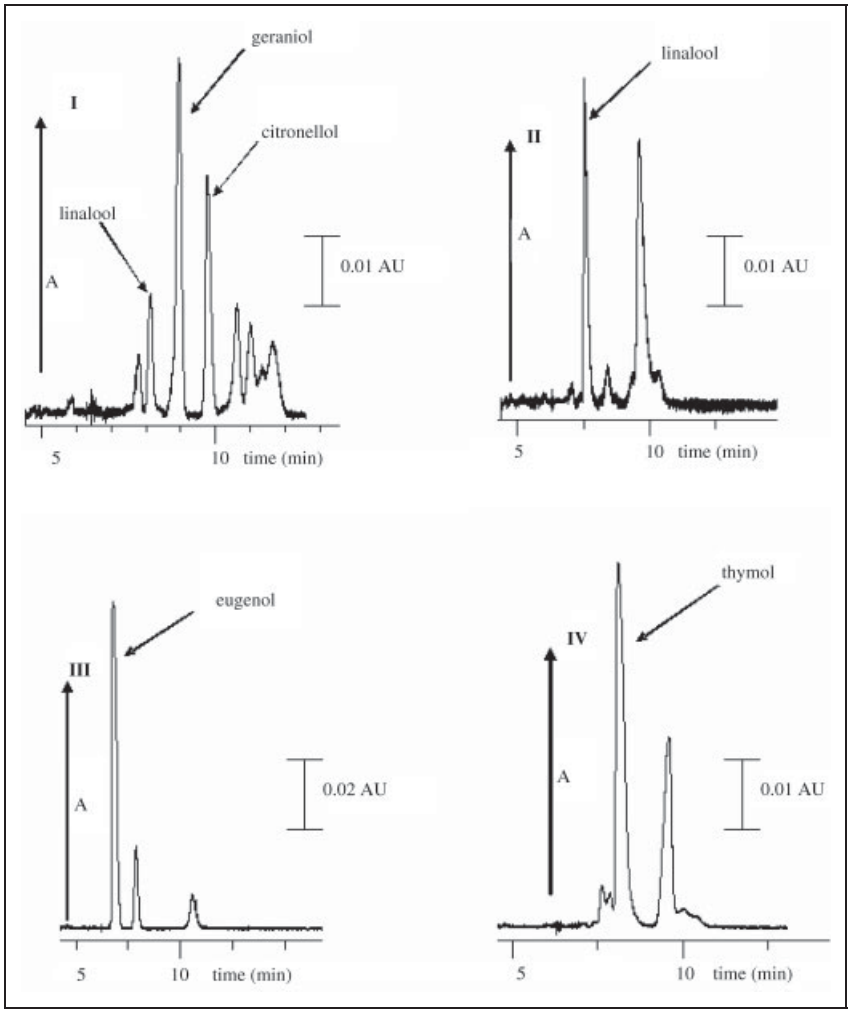


Fig. 2:
Electrophoreograms of commercial pharmacopoeial essential oils: (I) *Geranii etheroleum*, (II) *Lavandulae etheroleum*, (III) *Caryophylli floris etheroleum* and (IV) *Thymi etheroleum*

Table 2: Results of CE assay of major monoterpene alcohols in the pharmacopoeial essential oils and estimation of recoveries

Analyte	Found % (CE); (RSD %; n = 5)	Found % (GC); (RSD %; n = 5)	Added mg · l^{-1}	Found mg · l^{-1} (CE)	Recovery % (RSD %; n = 3)	t-Test*
Geranii etheroleum						
Linalool	11.52 (3.6)	12.18 (3.1)	100.0	98.4	98.4 (2.3)	2.348
Geraniol	21.93 (2.9)	22.82 (2.7)	100.0	96.8	96.8 (2.4)	2.047
Citronellol	37.56 (1.9)	38.74 (2.7)	100.0	98.9	98.9 (1.3)	1.818
Lavandulae etheroleum						
Linalool	41.88 (2.3)	43.13 (3.1)	100.0	96.6	96.6 (1.9)	1.518
Caryophylli floris etheroleum						
Eugenol	84.73 (1.6)	85.92 (1.2)	100.0	99.1	99.1 (1.3)	2.292
Thymi etheroleum						
Thymol	38.43 (1.7)	36.94 (2.9)	100.0	95.9	95.9 (2.1)	1.525

* At 95% confidence level; $n_A = n_B = 5$; $t_c = 2.571$ (critical value); n is the number of parallel samples analyzed

3. Experimental

3.1. Materials

The standards of eugenol (99%), linalool (98%), thymol (99.5%), geraniol (98%), citronellol (99%), citronellal (98%), farnesol (97%), sodium dodecyl sulphate (SDS), methanol and acetonitrile were obtained from Aldrich (USA), TAPSO (*N*-[tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid), 2-morpholinoethansulfonic acid (MES), 2-hydroxy-3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid (HEPPSO) and heptane puriss. p.a. (GC) were obtained from Fluka (Buchs, Switzerland), sodium hydroxide and TRIS (tris-(hydroxymethylamino)methane) were purchased from Lachema (Brno, Czech Republic). Commercial essential oils *Geranii etheroleum*, *Caryophylli floris etheroleum*, *Lavandulae etheroleum* and *Thymi etheroleum* were obtained from Kulich company (Hradec Králové/Říčany, Czech Republic). A Millipore Milli-Q RG ultra pure water was used throughout.

3.2. Equipment

A computer-controlled PrinCE 650 capillary electrophoresis system (Prince Technologies B.V., The Netherlands) equipped with UV-VIS HPLC spectrophotometric detector LAMBDA (Leonberg, Germany) and WinPrinCE software (as an integrated module of DAX data acquisition and analysis package) was used. The separation capillary was a fused silica tube (50 μm I. D., total length 75 cm, 42 cm effective length) maintained at 25 °C. The separations were performed at the applied voltage of 20 kV. Samples were injected hydrodynamically at a pressure of 50 mbar for 6 s. Detection was carried out at 200 nm. The capillary was cleaned daily by flushing with 0.1 M NaOH for 60 min and 60 min with deionized water. The capillary was rinsed with 0.1 M NaOH (2 min), deionized water (2 min) and buffer (4 min) after each separation run. These steps were very important to prevent adsorption of the analytes on the capillary walls. The PHM 220 (Radiometer, France) pH-meter with PHC2401-8 combined glass electrode calibrated with standard Radiometer buffers was employed for pH measurements.

GC analysis was performed on Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector. Data were recorded and analysed by DataApex CSW® 1.7 (Prague, Czech Republic) software. A Supelco Equity™-5 (poly-5% diphenyl/95% dimethylsiloxane, 30 m × 0.32 mm, 1.0 μm) capillary column was used. The injector and the detector were operated at 250 °C. The GC analysis was performed in the split mode (1 : 100). The column temperature was programmed from 75 °C (8.0 min) to 200 °C at 4 °C min^{-1}. Nitrogen (99.999%) was used as the carrier gas and the column head pressure was set at 100 kPa that corresponds to a column linear flow rate of 3.0 ml/min.

3.3. Electrolytes and sample preparation for CE

Generally, the background electrolyte solutions examined during optimization of the separation of the terpenoids were prepared by dissolving the corresponding substances in aqueous 10% (v/v) acetonitrile. The apparent pH of the aqueous-organic BGEs was adjusted with TRIS; the pH values were measured directly in the prepared BGEs. The calibration measurements and the MEKC analyses of real samples were carried out with the optimum electrolyte of pH 7.5. To prepare 50 ml of such electrolyte 0.26 g of TAPSO and 0.43 g of SDS were dissolved in 45 ml of aqueous 10% (v/v) acetonitrile (a 15-min sonication was applied), the pH 7.5 of this solution was adjusted by the addition of TRIS (typically 0.6 to 0.7 g was needed; the pH change was monitored by using a combined glass electrode) and the final volume of the electrolyte was set to 50 ml with aqueous 10% (v/v) acetonitrile. Stock solutions containing 1 g · l^{-1} of eugenol, thymol and geraniol and 2 g · l^{-1} of linalool and citronellol were prepared by dissolving the weighed amount in methanol. The final model mixture of analyte standards was prepared by mixing the stock solution with the background electrolyte (1 : 9). Calibration solutions were prepared by diluting the stock solutions with the background electrolyte (BGE); they contained 100 mg · l^{-1} of farnesol as internal standard. Commercial essential oils were dissolved in methanol (concentration 10 g · l^{-1} of each) and diluted (1 : 4) with the BGE. All solutions were degassed for 15 min in an ultrasonic bath and filtered through a Millipore Millex-LCR membrane filter (pore size 0.45 μm).

3.4. Calibration curves

The calibration curves of terpene alcohols were obtained with five standard solutions of various concentrations ranging from 50 to 200 mg · l^{-1} for eugenol, thymol and geraniol and from 100 to 400 mg · l^{-1} for linalool and citronellol. Farnesol (100 mg · l^{-1}) was used as internal standard.

3.5. Standard solutions for gas chromatography

Stock solutions of reference standards of linalool, citronellol, geraniol, eugenol and thymol (5.0 mg · ml^{-1} of each) were prepared by dissolving accurate weight of the compound (50 mg) in heptane (10 ml). The stock solutions were stored at 5 °C in a refrigerator and brought to room temperature immediately before use. The calibration solutions (containing 0.1–1.0 mg ml^{-1} of the analytes) were prepared freshly by diluting the stock solution with heptane in appropriate quantities with addition of citronellal as internal standard. The calibration solutions containing 0.5 mg · ml^{-1} of citronellal as internal standard were injected in triplicate into the gas chromatograph.

3.6. Comparative quantitative GC analysis of essential oils

A liquid sample of *Geranii etheroleum, Lavandulae etheroleum, Caryophylli floris etheroleum* or *Thymi etheroleum* (0.5 g) was diluted to 10 ml with heptane in a volumetric flask. A 100-μl portion of this diluted sample solution was mixed with 100 μl of internal standard (50 mg · ml^{-1} of citronellal in heptane) and the mixture was diluted to 10 ml with heptane to get the test solution. The test solution and a reference standard solution containing all the analytes and the internal standard (0.5 mg · ml^{-1} of each in heptane) were injected in triplicate and the mean values of the peak areas of the analytes and internal standard were evaluated by using the appropriate software. The content of the individual analytes was calculated according to Eq. (1)

$$\%(\text{analyte}) = \frac{A_t/A_{ist}}{A_r/A_{isr}} \cdot \frac{C_{st} \cdot F}{m \cdot D} \cdot 100 \qquad (1)$$

where A_t is the peak area of the analyte in the test solution , A_{ist} is the peak area of the internal standard in the test solution, A_r is the peak area of the analyte in the reference solution, A_{isr} is the peak area of the internal standard in the reference solution, C_{st} (g · ml^{-1}) is the concentration of the analyte in the reference solution, F is purity of the reference standard, D is dilution of the sample and m (g) is the weighed amount of the sample of the essential oil.

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References

Eckshlager K, Horsák I, Kodejš Z (1980) In: Vyhodnocování analytickch vsledkù a metod (Evaluation of analytical results and methods), SNTL Publishing House, Prague, p. 44.

Ehlers D, Nguyen T, Quirin KW, Gepard D (2001) Analysis of essential oils of basil – supercritical carbon dioxide extracts and steam distillates. Dtsch Lebensm Rundsch 97: 245–250.

Falqué E, Fernández E, Dubourdieu D (2001) Differentiation of white wines by their aromatic index. Talanta 54: 271–281.

Holland RD, Sepaniak MJ (1993) Qualitative analysis of mycotoxins using micellar-electrokinetic capillary chromatography. Anal Chem 65: 1140–1146.

Kim HJ, Kim K, Kim NS, Lee DS (2000) Determination of floral fragrances of *Rosa hybrida* using solid-phase trapping-solvent extraction and gas chromatography-mass spektrometry. J Chromatogr A 902: 389–404.

Li G, Locke DC (1996) Nonionic surfactants for improving resolution of the priority pollutant phenols by micelle-modified capillary electrophoresis. J Chromatogr A 734: 357–365.

Ney I, Jaeger E, Herderich M, Schreier P, Schwab W (1996) Analysis of phosphorylated terpenes by electrospray ionization LC-MS/MS. Phytochem Anal 7: 233–236.

Nishi H, Terabe S (1990) Application of micellar-electrokinetic chromatography to pharmaceutical analysis. Electrophoresis 11: 691–701.

Ochocka RJ, Marzec A, Lamparczyk H (1992) Fluorescence enhancement of two terpenes commonly present in essential oils. J Pharm Biomed Anal 10: 809–812.

Ogawa CA, Diagone CA, Lancas FM (2002) Separation of monoterpenes in orange essential oil by capillary liquid chromatography and micellar electrokinetic chromatography. J Liq Chromatogr Relat Technol 25: 1651–1659.

Otsuka K, Terabe S (1996) In: Atria KD (ed.) In capillary electrophoresis guidebook, Humana Press, Hertfordhire, UK, p. 125–155.

Paúl JP, Alfonso MJP, Negueruela AV, Sanz J (2002) Analysis by gas chromatography-mass spectrometry of the volatile components of *Ageratina adenophora* Spreng., growing in the Canary Islands. J Chromatogr A 947: 327–331.

Ph. Eur. – European Pharmacopoeia (2002), 4th ed., Council of Europe, Strasbourg.

Píry J, Príbela A, Ďurčanská J, Farkaš P (1995) Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods. Food Chemistry 54: 73–77.

Porter AEA, Griffiths DW, Robertson GW, Sexton R (1999) Floral volatiles of the sweet pea *Lathyrus odoratus*. Phytochemistry 51: 211–214.

Rapparini F, Baraldi R, Facini O (2001) Seasonal variation of monoterpene emission from *Malus domestica* and *Prunus avium*. Phytochemistry 57: 681–687.

Rodrigues MRA, Caramao EB, Arce L, Rios A, Valcarcel M (2001) Use of cyclodextrins for the separation of monoterpene isomers by micellar electrokinetic capillary chromatography. J Microcolumn Sep 13: 293–299.

Rodrigues MRA, Caramao EB, Arce L, Rios A, Valcarcel M (2002) Determination of monoterpene hydrocarbons and alcohols in *Majorana hortensis* Moench by micellar electrokinetic capillary chromatography. J Agric Food Chem 50: 4215–4220.

Rohloff J (2002) Volatiles from rhizomes of *Rhodiola rosea* L. Phytochemistry 59: 655–661.

Rojas LB, Usubillaga A, Galarraga F (1999) Essential oil of *Coespeletia timotensis* Cuatrec. Phytochemistry 52: 1483–1484.

Terabe S, Otsuka K, Ichikawa K, Tsuchiya A, Ando T (1984) Electrokinetic separations with micellar solutions and open-tubular capillaries. Anal Chem 53: 111–113.

Venskutonis PR (1997) Effect of drying on the volatile constituents of thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.). Food Chemistry 59: 219–227.

Verzera A, Trozzi A, Cotroneo A, Lorenzo D, Dellacassa E (2000) Uruguayan essential oils. 12. Composition of Nova and Satsuma mandarin oils. J Agric Food Chem 48: 2903–2909.