Journal of Chromatography A, 1216 (2009) 2768–2775

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Fast gas chromatography characterisation of purified semiochemicals from essential oils of *Matricaria chamomilla* L. (Asteraceae) and *Nepeta cataria* L. (Lamiaceae)

Stéphanie Heuskin[a,c,*], Bruno Godin[a], Pascal Leroy[b], Quentin Capella[b], Jean-Paul Wathelet[c], François Verheggen[b], Eric Haubruge[b], Georges Lognay[a]

[a] Department of Analytical Chemistry, Gembloux Agricultural University, Passage des Déportés 2, B-5030 Gembloux, Belgium
[b] Department of Functional and Evolutionary Entomology, Gembloux Agricultural University, Passage des Déportés 2, B-5030 Gembloux, Belgium
[c] Department of General and Organic Chemistry, Gembloux Agricultural University, Passage des Déportés 2, B-5030 Gembloux, Belgium

ARTICLE INFO

Article history:
Available online 7 October 2008

Keywords:
Aphids
Matricaria chamomilla
Nepeta cataria
Fast GC
Method validation
E-β-Farnesene
β-Caryophyllene
Nepetalactone
Fractionation

ABSTRACT

The chemical composition of *Matricaria chamomilla* L. and *Nepeta cataria* L. essential oils was determined by GC–MS on an apolar stationary phase by comparison of the characteristic fragmentation patterns with those of the Wiley 275L database. The GC–MS chromatograms were compared with those obtained by fast GC equipped with a direct resistively heated column (Ultra Fast Module 5% phenyl, 5 m × 0.1 mm, 0.1 μm film thickness). Analytical conditions were optimised to reach a good peak resolution (split ratio = 1:100), with analysis time lower than 5 min *versus* 35–45 min required by conventional GC–MS. The fast chromatographic method was completely validated for the analysis of mono- and sesquiterpene compounds. Essential oils were then fractionated by column chromatography packed with silica gel. Three main fractions with high degree of purity in *E*-β-farnesene were isolated from the oil of *M. chamomilla*. One fraction enriched in (*Z*,*E*)-nepetalactone and one enriched in β-caryophyllene were obtained from the oil of *N. cataria*. These semiochemical compounds could act as attractants of aphid's predators and parasitoids.

1. Introduction

Since a few years, essential oils and their constituents with semiochemical properties are more and more used for insect control in integrated pest management programs to encounter or drastically reduce the pesticides treatments [1–4]. There are many advantages for isolating semiochemicals from plant matrixes, like the essential oil fractionation technique, rather than by a chemical synthesis: the compounds of interest are natural molecules, the fractionation process is fast and simple to implement and the production costs are low. For supporting this technique, it is necessary to work with state-of-the-art analytical instrument for the determination and the quantification of products, like fast gas chromatography. Indeed, it is particularly suitable when a large number of fractions have to be checked.

In the present study, the main goals consist in isolating aphid pheromones molecules from a plant source and formulating them to attract aphid predators and/or parasitoids on the infested fields. *E*-β-Farnesene (EBF) and (*Z*,*E*)-nepetalactone are respectively, the alarm and the sexual pheromones of many aphids species [5–7]. Moreover, β-caryophyllene is a molecule of interest having biological activity against aphid reproduction [2] and was identified as the aggregation pheromone of the Asian lady beetle *Harmonia axyridis* [8]. One of the main interest of these compounds is that they could act as attractants and oviposition inductors of some aphid predators (*Episyrphus balteatus* De Geer (Diptera: Syrphidae)) and parasitoids (*Aphidius ervi* Haliday (Hymenoptera: Braconidae)) [9–13]. The essential oil of *Matricaria chamomilla* L. (Asteraceae), popularly known as German chamomile (other synonyms: *Matricaria recutita* L. and *Chamomilla recutita* L.), was reported to contain a high proportion of *E*-β-farnesene. The percentage of this compound can vary in function of the cultivar, the chemotype and the manufacturing process [14], and the part of the plant [15,16]. (*Z*,*E*)-Nepetalactone and β-caryophyllene are present as the major constituents in the essential oil of *Nepeta cataria* L. (catnip oil) (Lamiaceae) [17,18]. Another isomer of nepetalactone (*E*,*Z*)-form, is present in small proportions in the catnip essential oil and is reported to be repellent to cockroaches [18].

The fractionation of these essential oils by liquid column chromatography, with pentane as elution solvent, is a fast and simple

* Corresponding author at: Department of Analytical Chemistry, Gembloux Agricultural University, Passage des Déportés 2, B-5030 Gembloux, Belgium.
Tel.: +32 81 62 22 18; fax: +32 81 62 22 16.
E-mail address: heuskin.s@fsagx.ac.be (S. Heuskin).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.09.109

separation method to isolate groups of components (monoterpenes, sesquiterpenes, oxygenated compounds, etc.). The solvent, with a low-bowling point, could be evaporated rapidly without significant loss of compounds of interest. The isolation of *E*-β-farnesene from essential oil of *M. chamomilla* by this technique was reported by Bungert et al. [19]. The method proposed by these authors combined adsorption chromatography and argentation HPLC and is quite laborious. The procedure we describe here is faster and leads to adequate *E*-β-farnesene purification for performing biological tests.

As for most volatile terpenoids of essential oils, *E*-β-farnesene, nepetalactones and β-caryophyllene are generally analysed by conventional GC and GC–MS, but GC analytical methods are still time consuming, principally for the analysis of a great number of essential oil fractions. The necessity for fast GC methods is growing for routine analyses with repeatable and reproducible results. The efficiency of the fast GC technique with a direct resistively heated column (ultra fast module-GC) was demonstrated for the analyses of various types of samples: essential oils, pesticides, lipids, etc. [20–22].

The present research describes a completely validated fast GC method for the analysis and the quantification in less than 5 min of different mono- and sesquiterpenes. The method proposed herein could be easily transposed to other components of essential oils. The fast method was validated in term of repeatability, reproducibility, linearity, accuracy, selectivity and limits of detection (LOD)/quantification (LOQ). The sample capacity and the column efficiency were also evaluated respectively, with the evolution of the number of theoretical plates in function of the amount of sample injected, and with the Van Deemter plots. The gain of analytical time is about of a factor ten compared with conventional GC, with an optimal peak resolution. The original GLC method described in the present paper allows very high throughput and is of particular interest for the study of slow release formulations (ongoing investigations).

2. Experimental

2.1. Chemicals and materials

Essential oil of *M. chamomilla* was purchased from Vossen & Co. (Brussels, Belgium) and was originated from Nepal (lot no. CHA06MI0406). Essential oil of *N. cataria* was purchased from APT-Aromatiques (Saint-Saturnin les Apt, France) and was originated from France (lot no. 18007).

E-β-Farnesene from chemical synthesis was kindly supplied by Dr. S. Bartram and Prof. W. Boland (Max Planck Institute for Chemical Ecology, Jena, Germany). β-Caryophyllene, *n*-butylbenzene, α-pinene and longifolene as reference compounds were purchased from Sigma–Aldrich (Bornem, Belgium). The purity of the references was determined by fast GC. Solution of each compound was prepared in *n*-hexane at an approximate concentration of 1 μg/μl. Three replicates were analysed. A list of reference compounds mean purities, with standard deviations (SDs) and relative standard deviations (RSDs) is given in Table 1.

Table 1
Purity of reference compounds.

Compound	Mean purity (%)	SD	RSD (%)
E-β-Farnesene	98.17	0.0009	0.10
β-Caryophyllene	94.67	0.0071	0.75
Longifolene	98.01	0.0003	0.03
n-Butylbenzene	100.00	0.0000	0.00
Limonene	100.00	0.0000	0.00
α-Pinene	100.00	0.0000	0.00

2.2. GC–MS analyses

Conventional GC–MS analyses were carried out on a Thermo Trace GC Ultra coupled with a Thermo Trace MS Finnigan mass-selective detector (Thermo Electron Corp., Interscience, Louvain-la-Neuve, Belgium) and equipped with an Optima 5 MS (Macherey-Nagel) capillary column (30 m × 0.32 mm I.D., 0.25 μm film thickness). The oven temperature program was initiated at 40 °C, held for 5 min then raised first at 5 °C/min to 230 °C, raised in a second ramp at 30 °C/min to 280 °C with a final hold at this temperature for 5 min. Carrier gas: He, constant flow rate of 1.5 ml/min. Injection volume: 1 μl. Split ratio = 1:20. Injection temperature: 240 °C. Interface temperature: 280 °C. MS detection was performed with electron impact (EI) mode at 70 eV by operating in the full-scan acquisition mode in the 35–350 amu range. The identification of the volatile compounds was performed by comparing the obtained mass spectra with those from the Wiley 275L spectral library.

Retention indices (*I*) were determined relative to the retention times of a series of *n*-alkane standards (C9–C30, Sigma, 0.025 μg/μl in *n*-hexane), measured under the chromatographic conditions described above, and compared with literature values [23].

2.3. Fast GC analyses

Fast GC analyses were carried out on a Thermo Ultra Fast Trace GC gas chromatograph operated with a split/splitless injector and a Thermo AS 3000 autosampler (Thermo Electron Corp.). The GC system is equipped with an Ultra fast module (UFM) incorporating a direct resistively heated column (Thermo Electron Corp.): UFC-5, 5% phenyl, 5 m × 0.1 mm I.D., 0.1 μm film thickness. The following chromatographic conditions are those of the fast GC validation method. Temperature program of UFM: initial temperature at 40 °C, held for 0.1 min, ramp 1 at 30 °C/min to 95 °C, ramp 2 at 35 °C/min to 155 °C, ramp 3 at 200 °C/min to 280 °C, held for 0.5 min. Injection temperature: 240 °C. Injection volume: 1 μl. Carrier gas: He, at constant flow rate of 0.5 ml/min. Split ratio = 1:100. Detection: the GC unit has a high-frequency fast flame ionization detection (FID) system (300 Hz), at 250 °C. H_2 flow: 35 ml/min; air flow: 350 ml/min; makeup gas flow (N_2): 30 ml/min. Data processing was by Chromcard software (Version 2.3.3).

2.4. NMR spectra

All NMR spectra were recorded on Varian VNMR system (100, 400 and 600 MHz) spectrometers operating at 14.1 T or 9.4 T for 20 μl of sample diluted in 700 μl of $CDCl_3$. The signal of solvent was used as internal reference of chemical displacement (^{1}H: 7.26 ppm, ^{13}C: 77.16 ppm).

2.5. Essential oils fractionation and purification

Liquid column chromatographic separation of essential oils was used to obtain fractions enriched in compounds of interest. For that purpose, 1 ml (0.9306 g for *M. chamomilla* and 0.9525 g for *N. cataria*) of essential oil was fractionated over 11 g of silica gel G60 (70–230 mesh: ref. no. 815330.1, from Macherey-Nagel) previously dried during 16 h at 120 °C and packed in a glass column (15 mm I.D.) with glass wool plug at the bottom. Essential oil of *M. chamomilla* was eluted with 125 ml *n*-pentane to yield five fractions respectively, of 25, 10, 45, 25 and 20 ml. Essential oil of *N. cataria* was first eluted with 125 ml *n*-pentane to yield four fractions respectively, of 20, 40, 50 and 15 ml, followed by a second elution step with 70 ml *n*-pentane:diethyl ether (80:20) leading to two fractions