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

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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.

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

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 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 argenta-tion 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.

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].

Table1 : 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

2.3. 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

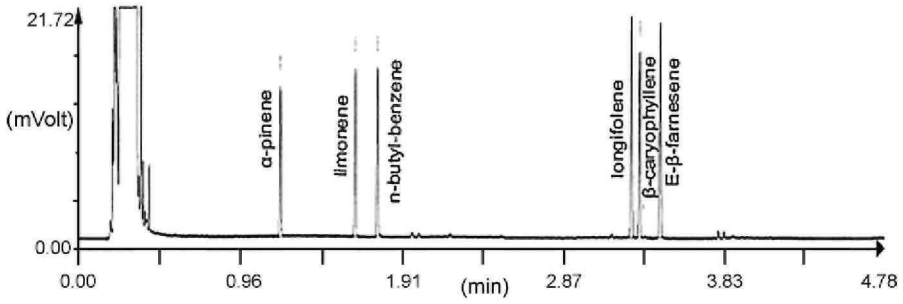
All NMR spectra were recorded on Varian VNMR system (100, 400 and 600 MHz) spectrometers operating at 14.1 T or 9.4T for 20 µl of sample diluted in 700 µl of $CDCl_3$. The signal of solvent was used as internal reference of chemical displacement (^1H*:* 7.26 ppm, ^{13}C: 77.16 ppm).

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 of 35 ml each. Fifty microlitres of different fractions were diluted 30 times in *n*-hexane prior to GC-MS and fast GC analyses.

Solvent-free purified compounds were obtained after evaporation of solvents from fractions at atmospheric pressure and at 40°C with a Büchi rotatory evaporator without vacuum. Solvent-free fractions were diluted in *n*-hexane and analysed at fast GC.

Fig. 1.: *Chromatogram of reference compounds and internal standards analysed with optimised fast GC method. For analysis conditions, see text.*



2.6. Method validation

The validation of the method for the quantification of volatile compounds (mono- and sesquiterpenes) was done for two ranges of concentrations (Range 1: 0.008-0.100 µg/µl, Range 2: 0.080-1.000 µg/µl). Longifolenewas used as internal standard for the sesquiterpene components (*E*-β-farnesene and β-caryophyllene). *n*-Butylbenzene was used as internal standard for the monoterpenes (limonene and α-pinene). This molecule has close molecular weight and is chromatographically well resolved in the prementioned conditions. Calibration curves were obtained by plotting the ratio of analysed peak area/I.S. peak area, *versus* the concentration ratio (analysed component/I.S.). For the first range, the concentration of longifolene I.S. was at 0.0497 µg/µl and the concentration of *n*-butylbenzene I.S. was at 0.0534 µg/µl. For the second range, the longifolene and the *n*-butylbenzene concentrations were at 0.4966 µg/µl and 0.5340 µg/µl, respectively. For the calibration curves of each component, six standard solutions from 0 µg/µl to 0.100 µg/µl in *n*-hexane (Range 1) and six standard solutions from 0 µg/µl to 1.000 µg/µl in *n*-hexane (Range 2) were used as data points. Each of the 12 concentration levels were analysed in triplicate. The calibration curves were calculated using the method of least squares fit analysis. The linearity was considered satisfactory when correlation coefficient (r^2) was higher than 0.996 [24].

The accuracy of the method was expressed as the bias (%) between the assigned value and the measured value. The accuracy was judged satisfactory when comprised between 90% and 110% [24].

The precision was evaluated by the determination of the repeatability and the reproducibility. To measure the repeatability, 10 replicates of a sample were analysed at 0.05 µg/µl and 0.5 µg/µl, for the first and the second range of concentrations respectively, on the same day by one analyst (n = 10). To define the reproducibility, 10 replicates at 0.05 µg/µl and 10 at 0.5 µg/µl, were analysed five times on 5 days (n = 50). Maximum allowed values (%) for repeatability and reproducibility were depending on the concentration (AOAC norm, 2006) (Range 1 : repeatability of 8%, reproducibility of 16%; Range 2: repeatability of 6%, reproducibility of 12%).

The limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit ($LOD = 3SD_{blank}$) for eight replicates of blank. The limit of quantification (LOQ) was arbitrarily set at 2LOD.

The selectivity of the method was defined with the selectivity factor *(α)* between the two nearest peaks (longifolene and β-caryophyllene):

$$\alpha = \left(\frac{t'_{R\beta-\text{caryophyllene}}}{t'_{\text{R longifolene}}} \right),$$

where t_R' are the reduced retention times.

2.7. Calculation of direct resistively heated column (UFM) efficiency

The analytical performances of the UFM column were determined with the calculation of theoretical plates in function of the quantity of component injected in the chromatographic column. For each compound, 12 quantities were injected in triplicate from 0.08 ng to 50 ng range. The mean value of the number of theoretical plates for the three replicates was plotted in function of the quantity injected on the column.

The height of theoretical plates was calculated for each component in function of the carrier gas velocity. 0.5 ng of all components were injected on the column for 11 velocities ranging from 6.70 cm/s to 66.49 cm/s. Three replicates were realised. The mean value of the height of theoretical plates was plotted in function of the carrier gas velocity.

3. Results and discussion

The first part of this study concerns the validation of the fast GC analytical method for the quantification of monoterpene and sesquiterpene hydrocarbons with the calculation and study of theoretical plates, while the latter part deals with the purification of semiochemical compounds from essential oils of *M. chamomilla* and *N. cataria.*

3.1. Fast GC analytical method validation

Fig. 1 shows the chromatogram obtained with the fast GC analytical method for the reference compounds (α-pinene, limonene, *E*-β-farnesene and β-caryophyllene) and the internal standards, *n*-butylbenzene and longifolene for monoterpenes and sesquiterpenes, respectively. The concentrations of these compounds were at 0.500 µg/µl in *n*-hexane. Another solution of reference compounds was analysed at 0.050 µg/µl in *n*-hexane. In each case, good separation of analytes was achieved with acceptable peak resolution and symmetry within a total runtime of 5 min, with a split ratio of 1:100.

The linearity of the method is summarised in Table 2 which shows calibration data and detection limits for reference compounds in the two working ranges (6 points from 0.008 µg/µl to 0.100 µg/µl and 6 points fom 0.080 µg/µl to 1.000 µg/µl;*n* = 3), as well as the accuracy of the calibration curves. The linearity of the calibration curves was validated with the r^2 coefficients largely upper than 0.996 and with the Grubbs's test where reduced residual are lower than 2.754 in absolute value [25]. The results show that within the indicated concentration ranges, there was a good correlation between peak area and concentration of compounds. The accuracy of calibration curves was dependent of compounds and ranges of concentration. The observed values, close to 100%, are comprised in the theoretical acceptable limits (90-110%), and give a very strong accuracy for each compound in the two ranges of concentrations. The LOD and LOQ values are expressed in pg. They were calculated accounting for the dispersibility of eight blank replicates. As shown in Table 2, the values are dependent of the ranges of concentration and the compounds. The lowest values of LOD and LOQ are for β-caryophyllene with 0.74 pg and 1.48 pg, respectively.

Table 3 shows the precision of the method with repeatability and reproducibility for each compound at two concentrations (mean concentration of each range). The RSDs for repeatability were lower than the values of the AOAC norm which requires 8% and 6%, for the first and the second range of concentrations, respectively. For the reproducibility, the RSDs were lower than 16% and 12% for the ranges 1 and 2. These RSD values are very good and show strong repeatability and reproducibility of the method for each reference compounds, the worst being α-pinene with RSDs repeatability at 2.07% and 1.78%, for ranges 1 and 2 respectively, and with RSDs reproducibility at 3.48% and 7.51% for ranges 1 and 2. Considering these results, the precision of the fast GC method was widely satisfactory at both high and low concentrations.

The selectivity of the method is expressed inTable 4 as the selectivity factor *(α)* between the two nearest peaks, longifolene and β-caryophyllene, presented with SDs and RSDs. The selectivity was good with α at 1.016 for both ranges of concentrations.

3.2. Analytical performances of UFM column

Fig. 2 shows the evolution of the number of theoretical plates in function of the quantity of compounds injected on the UFM column. The number of plates is higher for sesquiterpenes than for monoterpenes, the retention times of sesquiterpenes being longer. As it can be seen, the maximum theoretical plate number is constant until a threshold amount of sample. It was determined that for the monoterpenes, quantities of up to 10 ng can be accomodated without affecting chromatographic resolution, while for the sesquiterpenes the same is true for values below 1 ng. The injection of higher quantities led to fronting peak distorsions.

The efficiency of analyses in function of velocity is shown in Fig. 3 where Van Deemter plots (derived from experimental data) were drawn, with the height of theoretical plates in function of carrier gas velocity (cm/s) for monoterpene and sesquiterpene reference compounds. The optimal height of a theoretical plate corresponded at the minimum value of *H* in the curve : H_{min}. For the two groups of components (monoterpenes and sesquiterpenes), the H_{min} value is obtained at a velocity of 35.86 cm/s. H_{min} is at 0.036 mm, 0.017 mm and 0.014 mm, for α-pinene, limonene and *n*-butylbenzene, respectively. The validation of the method was realised at a velocity of 43.94 cm/s, near optimal velocity, which allows faster analyses without affecting the efficiency of the separation.

3.3. Analysis of essential oils

Essential oils of *M. chamomilla* and *N. cataria* were analysed by GC-MS (5 µg/µl in *n*-hexane) to determine their compositions in compounds of interest: *E*-β-farnesene, β-caryophyllene and (*Z,E*)-nepetalactone.

The essential oil composition of *M. chamomilla* was dominated by monoterpene and sesquiterpene hydrocarbons: 16 hydrocarbon sesquiterpenes (59.00%), 9 oxygenated sesquiterpenes (31.63%), 7 hydrocarbon

monoterpenes (1.25%) and 3 oxygenated monoter-penes (0.41%). Through comparison of the retention index with those of the literature [23] and EI mass spectra of each peak with the library, it was possible to identify 41 individual components, representing 99.57% of the total amount (Table 5). The major peaks observed in the chromatogram were attributed to sesquiterpenes with *E*-β-farnesene (42.59%), (*E*,*E*)-α-farnesene (8.32%), germa-crene D (2.93%), bicyclogermacrene (1.99%), chamazulene (1.18%) and oxygenated sesquiterpenes with α-bisabololoxide A (21.2%), α-bisabolone oxide A (4.53%) and α-bisabolol oxide B (4.43%). Two sesquiterpenes representing 0.33% and 0.10%, respectively, remained unknown.

Fig. 2*.: Number of theoretical plates (N) in function of the quantity of compounds injected on the Ultra Fast Module column. (A) E-β-farnesene, (B) β-caryophyllene, (C) longifolene, (D) n-butyl-benzene, (E) limonene and (F) α-pinene.*

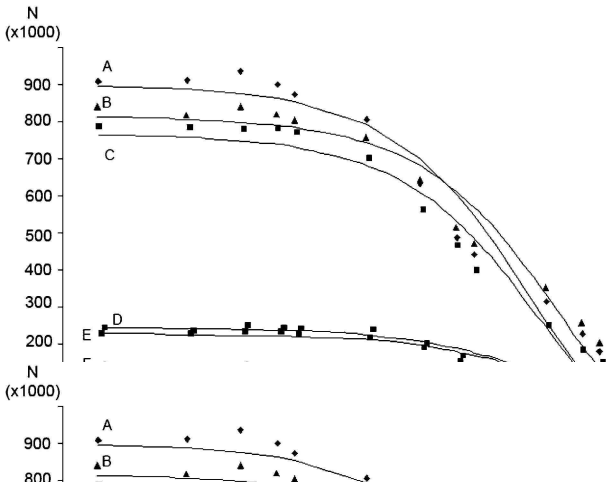


Fig. 3.*: Van Deemter plots for fast GC (A) α-pinene, (B) limonene, (C) n-butyl-benzene, (D) longifolene, (E) β-caryophyllene and (F) E-β-farnesene.*

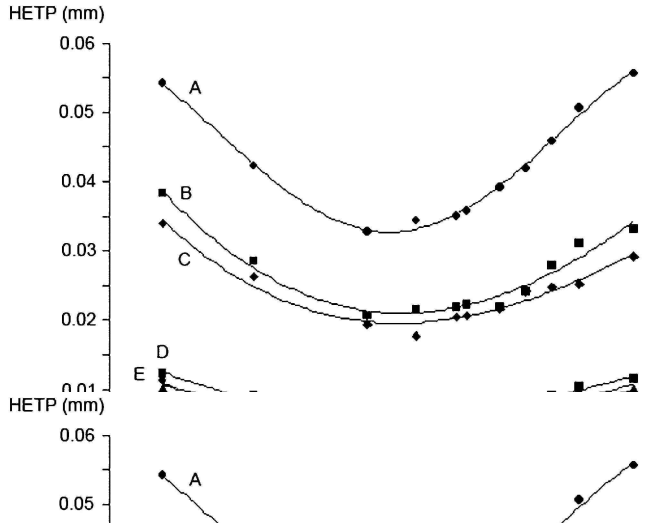


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Table 2**:** *Linearity data for the fast GC validation method.*

	***E*-β-Farnesene**		**β-Caryophyllene**		**Limonene**		**α-Pinene**	
Range (µg/µl)	0.008-0.100	0.080-1.000	0.008-0.100	0.080-1.000	0.008-0.100	0.080-1.000	0.008-0.100	0.080-1.000
Equation of the calibration curve	y = 0.9592*x*- 0.0028	y = 0.9558*x*+ 0.0053	y = 0.8381*x* + 0.0030	y = 0.8408*x*+ 0.0056	y = 0.9767*x*+ 0.0024	y = 0.9690*x*+ 0.0019	y = 0.8004*x* + 0.0012	y = 0.8097*x*-0.0013
r^2	0.9998	0.9999	0.9998	0.9999	0.9998	0.9999	0.9988	0.9993
Reduced residual (Grubb's test)	2.668	1.866	2.147	1.880	2.394	1.826	2.134	2.276
Accuracy of calibration curves (%)[a]	99.19	99.86	99.77	99.90	100.85	100.67	103.65	102.36
Internal standard	Longifolene	Longifolene	Longifolene	Longifolene	*n*-Butyl-benzene	*n*-Butyl-benzene	*n*-Butyl-benzene	*n*-Butyl-benzene
LOD (pg)	2.38	2.40	1.79	0.74	2.43	1.37	2.11	2.05
LOQ(pg)	4.76	4.80	3.58	1.48	4.86	2.74	4.22	4.10

[a] Bias (%) between the measured value and the theoretical value.

Table 3: *Precision of the fast GC method expressed as repeatability and reproducibility.*

	***E*-β-Farnesene**		**β-Caryophyllene**		**Limonene**		**α-Pinene**	
Concentration (µg/µl)	0.050	0.500	0.050	0.500	0.050	0.500	0.050	0.500
Repeatability (RSD, %)	1.16	0.70	0.43	0.12	0.70	0.42	2.07	1.78
Reproducibility (RSD, %)	3.00	2.82	0.89	0.81	1.98	1.89	3.48	7.51

Table 4: *Selectivity of the fast GC method.*

	Concentration range: 0.008-0.100 µg/µl	**Concentration range: 0.080-1.000 µg/µl**
Selectivity factor *(α)* between longifolene and β-caryophyllene	1.016	1.016
SD	0.001	0.001
RSD (%)	0.07	0.07

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Table 5: *Constituents of the essential oil of Matricaria chamomilla identified by GC-MS.*

No.	Components	Retention time (min)	Retention index (measured)	%
1	α-Pinene	9.92	922	0.03
2	Sabinene	11.38	965	0.04
3	6-Methyl-5-hepten-2-one	11.83	977	0.03
4	2-Pentylfuran	12.08	988	0.05
5	*p*-Cymene	13.14	1018	0.11
6	Limonene	13.34	1024	0.10
7	*trans*-β-Ocimene	13.74	1036	0.11
8	*cis*-β-Ocimene	14.08	1046	0.69
9	γ-Terpinene	14.36	1054	0.17
10	Artemesia ketone	14.44	1057	0.32
11	Artemesia alcohol	15.20	1079	0.06
12	Isoborneol	17.68	1153	0.03
13	4,8-Dimethylnona-3,8-dien-2-one	20.87	1262	0.04
14	*α*-Copaene	23.78	1365	0.04
15	β-Maaliene	23.88	1369	0.07
16	*α*-Isocomene	24.06	1376	0.26
17	β-Elemene	24.19	1382	0.07
18	Sativene	24.55	1397	0.04
19	*α*-Gurjunene	24.69	1403	0.04
20	β-Caryophyllene	24.89	1411	0.17
21	Aromadendrene	25.40	1433	0.07
22	***E*-β-Farnesene**	**25.95**	**1456**	**42.59**
23	Not identified sesquiterpene (MW: 204)	26.15	1465	0.10
24	**Germacrène D**	**26.47**	**1478**	**2.93**
25	β-Selinene	26.59	1483	0.22
26	(Z,*E*)-α-Farnesene	26.77	1491	0.83
27	**Bicyclogermacrene**	**26.85**	**1494**	**1.99**
28	**(*E*,*E*)-α-Farnesene**	**27.13**	**1506**	**8.32**
29	δ-Cadinene	27.48	1521	0.18
30	Sesquirosefuran	28.15	1549	0.18
31	Not identified sesquiterpene (MW: 204)	28.33	1157	0.33
32	*trans*-Nerolidol	28.39	1559	0.17
33	Dehydronerolidol	28.46	1562	0.09
34	Dendrolasin	28.63	1569	0.21
35	Spathulenol	28.71	1573	0.63
36	Globulol	28.85	1578	0.23
37	**α-Bisabololoxide B**	**30.52**	**1649**	**4.43**
38	**α-Bisabolone oxide A**	**31.10**	**1673**	**4.53**
39	**Chamazulene**	**32.08**	**1715**	**1.18**
40	**α-Bisabololoxide A**	**32.55**	**1735**	**21.16**
41	***cis*-ene-yne-Dicyclo ether**	**35.15**	**1802**	**5.94**
42	*trans*-ene-yne-Dicyclo ether	35.30	1807	0.99
43	(*E*)-Phytol	39.79	2107	0.23

The bold values consist in the major constituents of the essential oils.

Table 6 shows the composition of the *N. cataria* essential oil. Twenty components were identified (El-mass spectra and retention index comparison). Among them, 6 were oxygenated sesquiterpenes (77.17%), 2 hydrocarbon sesquiterpenes (10.53%), 9 hydrocarbon monoterpenes (2.84%) and 2 oxygenated monoterpenes (0.09%). The main identified constituents of this essential oil are 4aα,7α,7aα-nepetalactone ((Z,*E*)-nepetalactone or *4aS,7S*,7a*R*-nepetalactone) (73.27%), β-caryophyllene (9.72%), β-caryophyllene oxide (1.81%), *cis*-β-ocimene (1.64%) and 4aα,7β,7aα-nepetalactone (1.10%). Three isomers of nepetalactone were present in the essential oil of *N. cataria*, but could not be identified with absolute configuration certainty by GC-MS. The stereoisomery of (Z,E)-nepetalactone was confirmed by ^{1}H and ^{13}C NMR spectrometry (data not shown) and by comparison of the chemical ^{13}C displacements with those of the literature [26]. Ten minor compounds of the essential oil could not be identified with the spectral library and retention index.

The essential oils were then analysed by fast GC on an UFM column of the same polarity as in GC-MS (apolar stationary phase). The retention times of the components of interest from the essential oils were compared to those of the reference compounds. Figs. 4 and 5 report the patterns, respectively of the *M. chamomilla* and the *N. cataria* essential oils, under study analysed by GC-MS and fast GC, together with a list of their characteristic components. With conventional GC-MS, analysis time was about 40 min, while for fast GC it was less than 5 min, with the same chromatographic profile and similar resolution.

Table 6: *Constituents of the essential oil of Nepeta cataria identified by GC-MS.*

No.	Components	Retention time (min)	Retention index (measured)	%
1	α-Thujene	9.72	916	0.01
2	α-Pinene	9.92	922	0.05
3	Sabinene	11.37	965	0.10
4	β-Pinene	11.44	967	0.24
5	β-Myrcene	12.12	987	0.02
6	Limonene	13.34	1024	0.28
7	*trans*-β-Ocimene	13.74	1036	0.47
8	***cis*-β-Ocimene**	**14.08**	**1046**	**1.64**
9	Linalool	15.71	1095	0.05
10	α-Terpineol	18.47	1177	0.04
11	Not identified monoterpene (MW: 136)	19.09	1196	0.54
12	Not identified monoterpene (MW: 136)	19.78	1216	0.03
13	Not identified monoterpene (MW: 136)	19.88	1219	0.12
14	Not identified monoterpene (MW: 136)	20.74	1245	0.10
15	**(*4aα,7α,7aα*)-Nepetalactone**	**23.51**	**1353**	**73.27**
16	(4aα,7α,7aβ)-Nepetalactone	23.94	1371	0.44
17	**(*4aα,7β,7aα*)-Nepetalactone**	**24.07**	**1377**	**1.10**
18	Dihydronepetalactone	24.56	1397	0.46
19	**β-Caryophyllene**	**24.89**	**1411**	**9.72**
20	α-Humulene	25.77	1449	0.81
21	Not identified	27.82		7.82
22	Benzoate (Z)-3-hexen-l-ol	28.48	1551	0.07
23	**β-Caryophyllene oxide**	**28.87**	**1579**	**1.81**
24	Humulene epoxide II	29.45	1604	0.09
25	Not identified	32.46		0.06
26	Not identified	33.69		0.05
27	Not identified	33.76		0.03
28	Not identified	33.91		0.38
29	Not identified	34.00		0.13
30	6,10,14-Trimethyl-2-pentadecanone	34.59	1840	0.07

The bold values consist in the major constituents of the essential oils.

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***Fig. 4**.: Profiles of a Matricaria chamomilla essential oil analysed by GC-MS (a) and fast GC (b). For analysis conditions see text. List of the main components: (1) E-β-farnesene; (2) germacrene D; (3) bicyclogermacrene; (4) (E,E)-α-farnesene; (5) α-bisabolol oxide B; (6) α-bisabolone oxide A; (7) chamazulene; (8) α-bisabolol oxide A; (9) cis-ene-yne-dicycloether.*

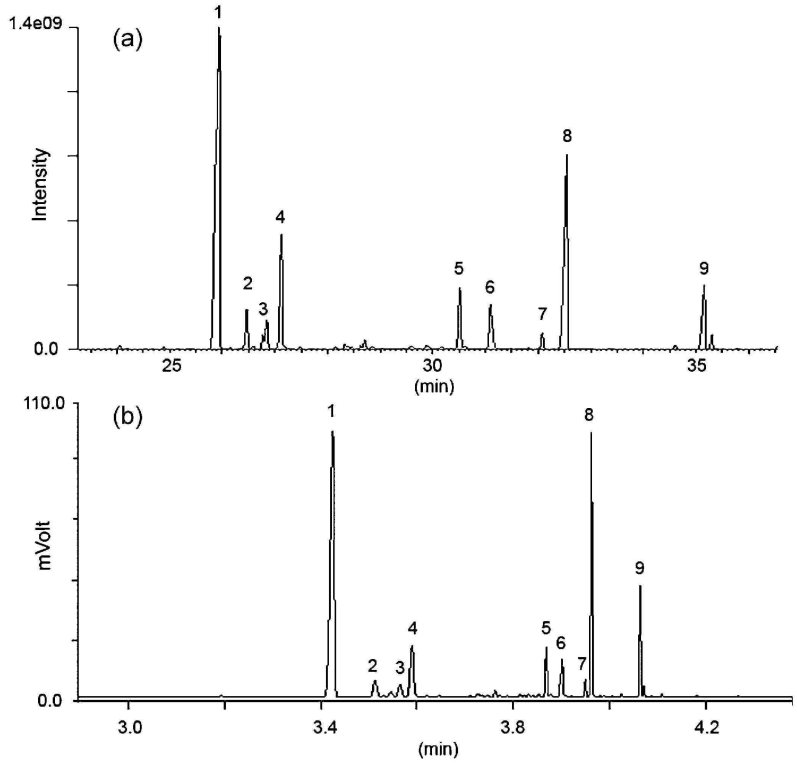

***Fig. 5**.: Profiles of a Nepeta cataria essential oil analysed by GC-MS (a) and fast GC (b). For analysis conditions see text. List of the main components: (1) (Z,E)-nepetalactone; (2) (E,Z)-nepetalactone; (3) β-caryophyllene; (4) unknown compound; (5) β-caryophyllene oxide.*

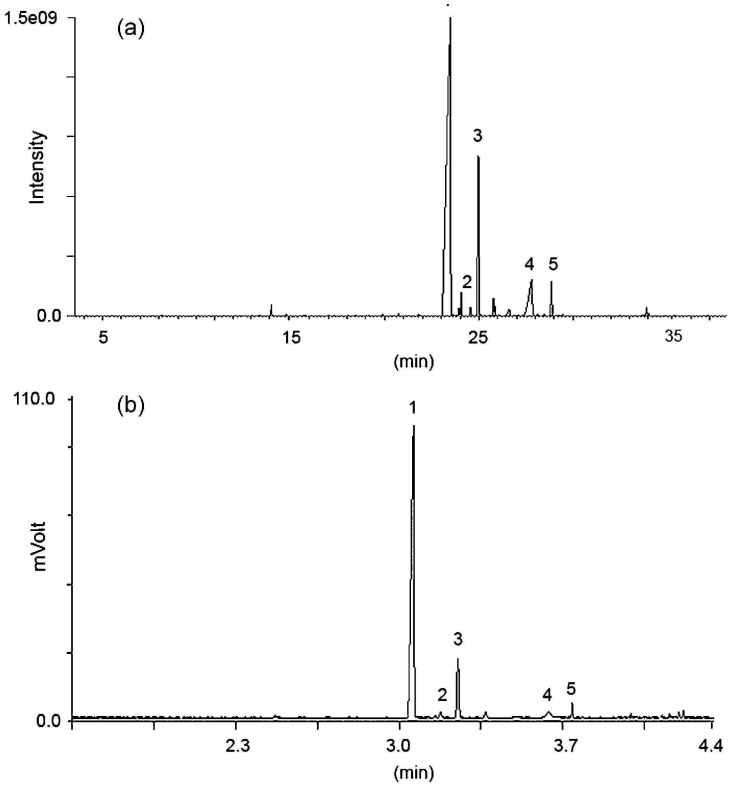

Table 7: *Major components of Matricaria chamomilla fractions (mean ± SD of triplicate).*

Matricaria chamomilla	**F1**		**F2**		**F3**		**F4**		**F5**	
	Composition (%)	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**
Sum of monoterpenes	4.38	1.32	2.73	0.58	0	0	0	0	0	0
E-β-Farnesene	51.79	0.68	74.90	2.28	79.61	0.06	76.78	1.50	68.99	2.16
(E,E)-α-Farnesene	3.98	0.40	9.20	1.91	14.34	0.81	20.86	0.87	25.22	1.63
Germacrene D	7.52	0.18	5.79	0.41	1.25	0.51	0	0	0	0
Chamazulene	0	0	0	0	0	0	0.69	0.66	4.52	2.44

Table 8 : *Major components of Nepeta cataria fractions (mean ± SD of triplicate).*

Nepeta cataria	**F1**		**F2**		**F3**		**F4**		**F5**		**F6**	
	Composition (%)	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**	**Composition (%)**	**SD (%)**
(Z,*E*)-Nepetalactone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	96.97	1.70	84.63	12.59
(*E*,Z)-Nepetalactone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.32	14.96	13.15
β-Caryophyllene	0.00	0.00	79.54	4.68	26.88	25.86	0.00	0.00	0.00	0.00	0.00	0.00
Caryophyllene oxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.19	0.39	0.10	0.18

3.4. Essential oils fractionation

Tables 7 and 8 present the mean composition (%) ±SD (n = 3) of *M. chamomilla* and *N. cataria* fractions recovered of elution on adsorption chromatography and solvent evaporation. The fractions were analysed on fast GC and the identification of peaks was realised by comparison of the different retention times. As it can be seen, each fraction differs from the original oil in its chromatographic profile and in the percentage of the constituents.

Five fractions were collected for *M. chamomilla* based on the evolution of *E*-β-farnesene percentage in function of *n*-pentane solvent elution volume. *E*-β-farnesene is present in each fraction and was always associated with (*E*,*E*)-α-farnesene. Germacrene D was also associated in small percentage to the two previous cited compounds in the three first fractions. F3 has the highest relative percentage of *E*-β-farnesene (79.61%) and, for this reason, is the most interesting in this study. The percentage of (*E*,*E*)-α-farnesene grows from F1 (3.98%) to F5 (25.22%), with a mean value of 14.34% in F3. Chamazulene was detected in fraction F4 (0.69%), but clearly appeared in F5 (4.52%) with a blue coloration of the fraction. This compound also served as coloured indicator during the fractionation process to stop the elution; the percentage of *E*-β-farnesene decreasing with the apparition of chamazulene.

The fractionation of *N. cataria* essential oil was reached in two elution steps. The first elution with *n*-pentane allowed the collection (four fractions from F1 to F4) of non-polar compounds such as β-caryophyllene in very high proportion as regards the percentage of this compound in the pure essential oil (9.72%). Therefore, β-caryophyllene is mainly present in fraction F2 at 79.54% purity. The same fractionation process on another catnip oil from the USA led to totally pure β-caryophyllene (>99.9% by fast GC) (data not shown). The second fractionation step (two fractions, F5 and F6) was realised with a more polar elution solvent mix (*n*-pentane 80%, diethylether 20%) to collect nepetalactone isomers. Fraction F5 was enriched to 96.97% in (Z,*E*)-nepetalactone, the aphid sexual pheromone, with caryophyllene oxide (2.19%) as associated compound.

Compared with the pure essential oils, fractions obtained by column chromatography are more concentrated in compounds of interest. This purification technique led to the isolation and the collection of various polarity compounds (monoterpenes, sesquiterpenes, oxygenated compounds, etc.) in a very fast way. Flash chromatography is currently developed to obtain purified compounds at higher scale and shows identical results.

It is noteworthy that such fractionation and fast GC analyses are powerful and simple methods to produce and analyse essential oils constituents.

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