Contribution to the study of semiochemical slow release formulations. Development of flash chromatographic methods

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RÉGION WALLONNE

Introduction

Essential oils and their constituents with semiochemical properties (communication signals between species) are more and more used for insect control in integrated pest management programs to encounter or drastically reduce the pesticides treatments. The main goals of the present study consist in isolating aphid semiochemical molecules from a plant source and formulating them to attract aphid predators and/or parasitoids on the infested fields. The essential oil of *Matricaria chamomilla* L. (Asteraceae) was reported to contain a high proportion of ***E*-β-farnesene**, the alarm pheromone of many aphids species. Another sesquiterpene ($C_{15}H_{24}$), **β-Caryophyllene**, identified as the aggregation pheromone of the Asian lady beetles *Harmonia axyridis* Pallas, is present as the major constituent in the essential oil of *Nepeta cataria* L. (Lamiaceae).

Aphids tritrophic system

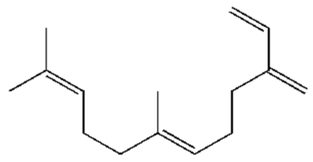
Host plant (*Vicia fabae* L.)

Phytophageous insects : aphids

Predator : larva of *Episyrphus balteatus* De Geer

Parasitoid : *Aphidius ervi* Haliday

E-β-farnesene



Alarm pheromone

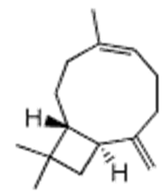
Aggregation phenomena of *Harmonia axyridis* Pallas

Larva of *Harmonia axyridis* Pallas

Adult of *Harmonia axyridis* Pallas

Aggregated colony

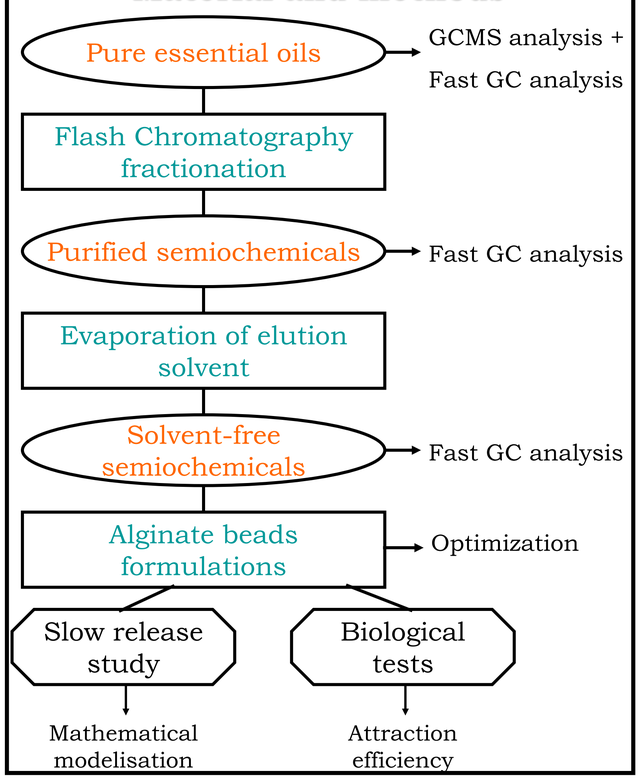
β-caryophyllene



Aggregation pheromone

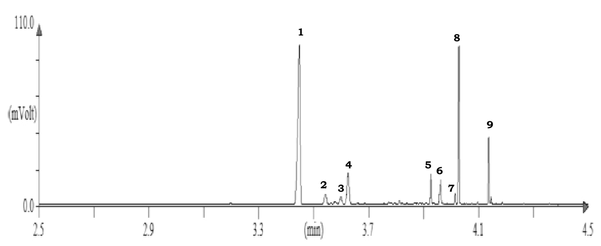
Experimental

Material and methods



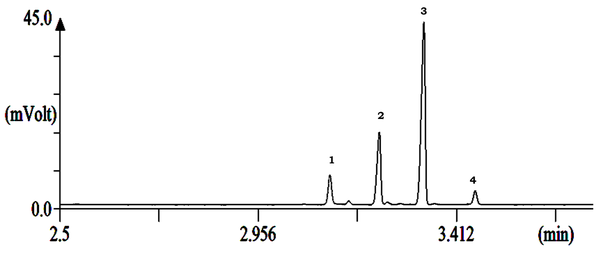
Essential oils analysis by Fast GC

Matricaria chamomilla



	Major compounds	Composition	Kovats indices
1	**E-β-farnesene**	**42.6 %**	1456
2	Germacrene D	2.9 %	1478
3	Bicyclogermacrene	2.0 %	1494
4	(E,E)-α-farnesene	8.3 %	1506
5	α-bisabolol oxide B	4.4 %	1649
6	α-bisabolone oxide A	4.5 %	1673
7	Chamazulene	1.2 %	1715
8	α-bisabolol oxide A	21.2 %	1735
9	Cis-en-in-dicycloether	5.9 %	1802

Nepeta cataria



	Major compounds	Composition	Kovats indices
1	(Z,E)-nepetalactone(*)	8.4 %	1353
2	(E,Z)-nepetalactone	22.5 %	1377
3	**β-caryophyllene**	**58.9 %**	1415
4	α-humulene	3.9 %	1465

(*) Stereoisomery confirmed by ^{1}H and ^{13}C NMR

Flash Chromatography fractionation

Matricaria chamomilla

Compounds	
Sum of monoterpenes	1.3 %
E-β-farnesene	**84.0 %**
Germacrene D	1.4 %
Bicyclogermacrene	1.4 %
(E,E)-α-farnesene	11.9 %

Nepeta cataria

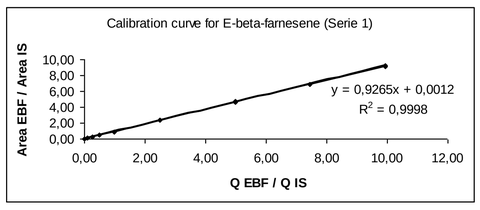
Compounds	
Sum of monoterpenes	1.5 %
β-caryophyllene	**97.4 %**
α-humulene	1.1 %

Validation by accuracy profile methodology

Example for E-β-farnesene

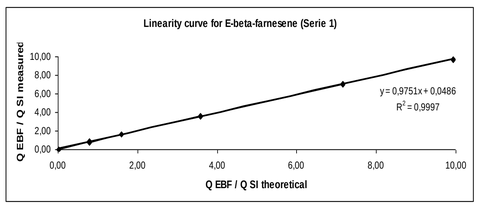
Calibration standards

Examples of calibration curves for E-β-farnesene with longifolene as internal standard



Validation standards

Back-calculation of the results ➔ linearity curves



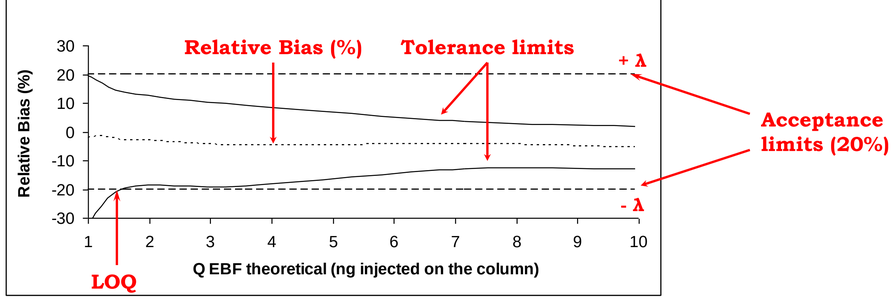
Accuracy Profile

ACCURACY = TRUENESS + PRECISION

Trueness : $\hat{u}_j$ = average amount back calculated by level of amount

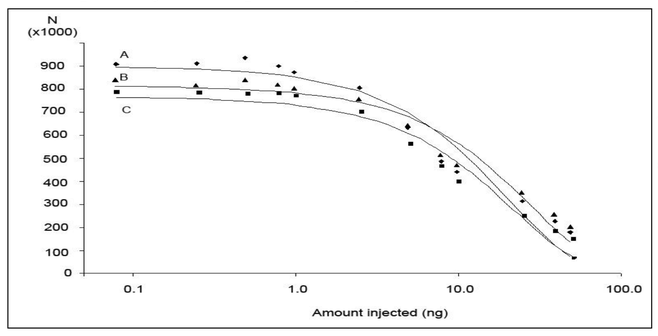
$$\textbf{Bias (\%)} = \hat{u}_j - \bar{X}_j$$

Precision : Repeatability variance ($\sigma^2_{W,j}$) + Inter-series variance ($\sigma^2_{B,j}$)



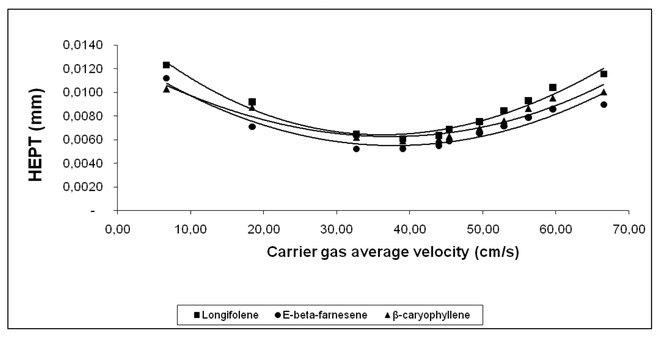
Analytical performances of the fast GC column

Number of theoretical plates (N) in function of the quantity of compounds injected on the column



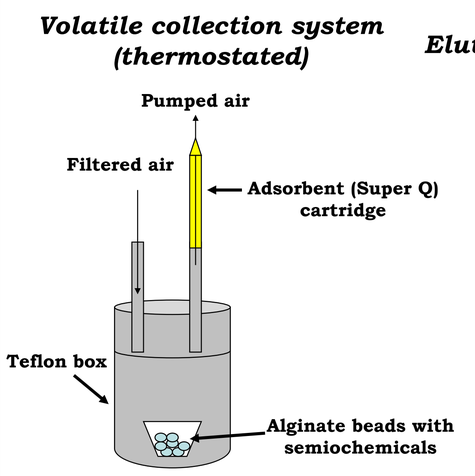
A : E-β-farnesene
B : β-caryophyllene
C : longifolene

Van Deemter plots for fast GC

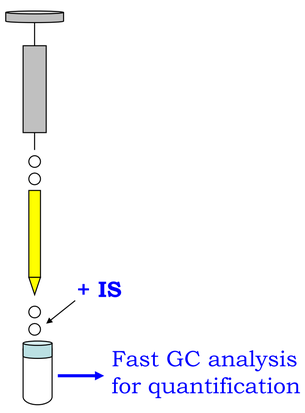


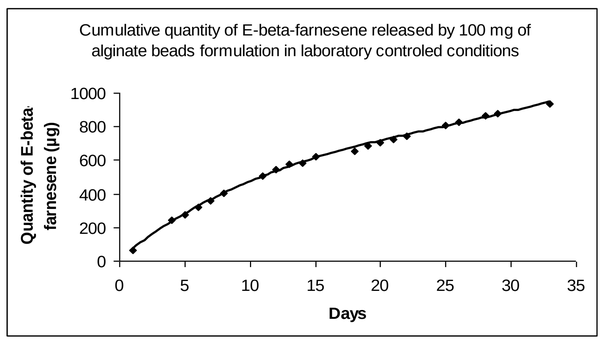
Study of slow release formulations

Volatile collection system (thermostated)



Elution of the cartridge





A mathematical modelisation (compartimentation model) of release is presently in study. The influence of physico-chemical parameters will be measured (T°, RH, diffusion coefficients, wind speed, light...) for E-β-farnesene and β-caryophyllene formulations

Conclusions

- Fast achievement of high purity semiochemicals by Flash Chromatography
- Fast GC method for characterization and quantification in less than 5 minutes
- Low limit of quantification calculated by accuracy profile method
- Good analytical performances of the fast column (high number of theoretical plates until 10 ng injected) (*Heuskin et al.*, 2009, J. of Chromatography A, 1216, pp 2768-2775)
- Simple and fast volatile collection system for the study of slow release formulations