# Étude chirale de l'huile essentielle d'*Artemisia herba alba* par dichroïsme circulaire vibrationnel et régression linéaire multiple

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# Plan

1- Introduction

2- Definitions

3- VCD spectrometer

4- Materials & Methods

5- Results

6- Conclusion

# Introduction

- Plants contain organic substances of various structure and use.
- Essential oils were widely studied through time.
- Our goal is the study the chirality of essential oils to determine the absolute configuration of the major chiral molecules.
- The vibrational circular dichroism (VCD) is a technique that was developed in the mid 70s.

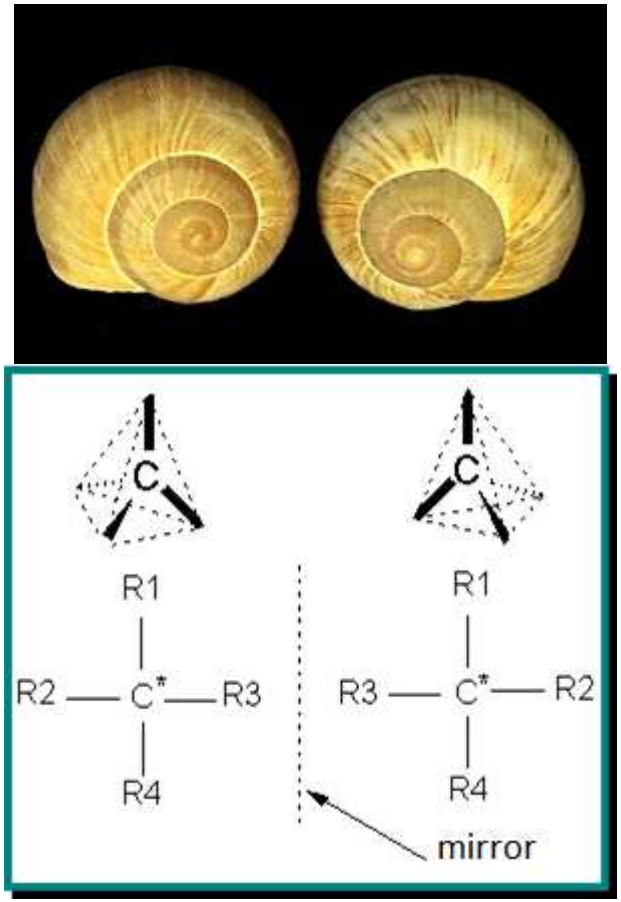
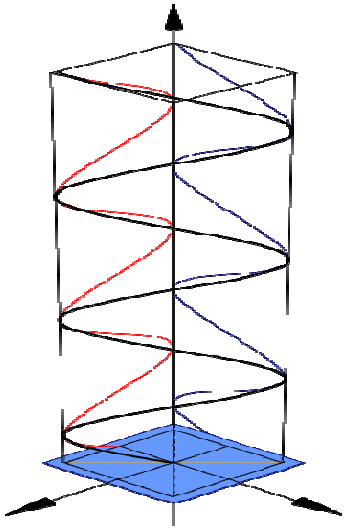


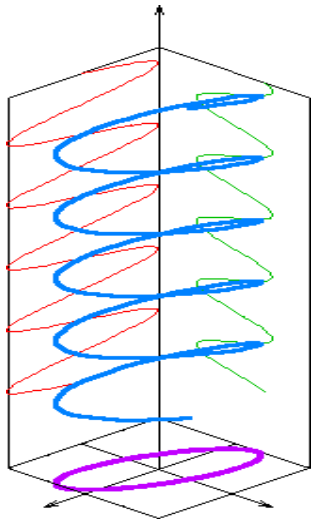

Fig 1. Chiral molecule

# Light Polarisation

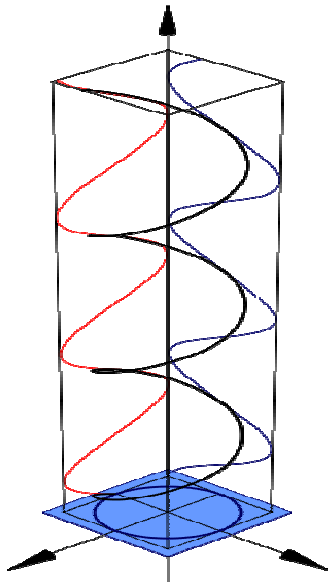
Light is an electromagnetic wave and has a polarization characteristic of the orientation of the electric field (or magnetic) in the plane perpendicular to the propagation.



Linear polarisation



Elliptic polarisation



Circular polarisation

# *Definitions*

## Circular Dichroism (CD)

A material has a circular dichroism if it absorbs differently left (LCP) and right (RCP) circularly polarised light.

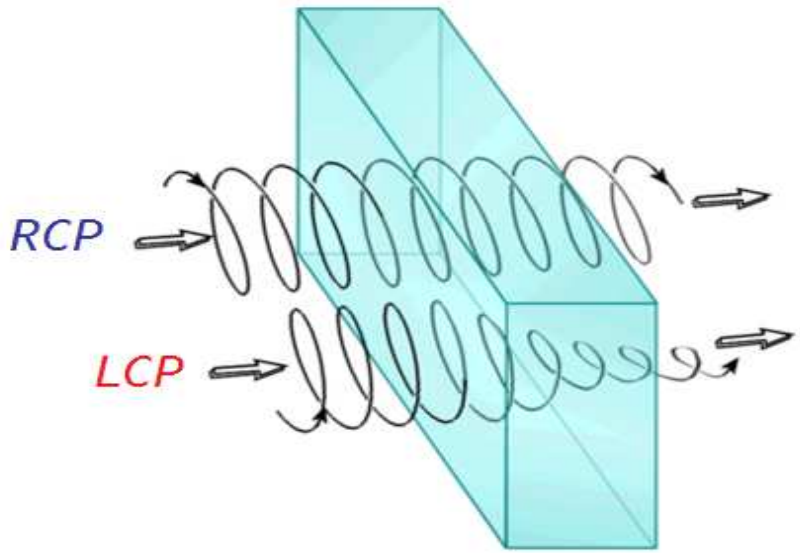
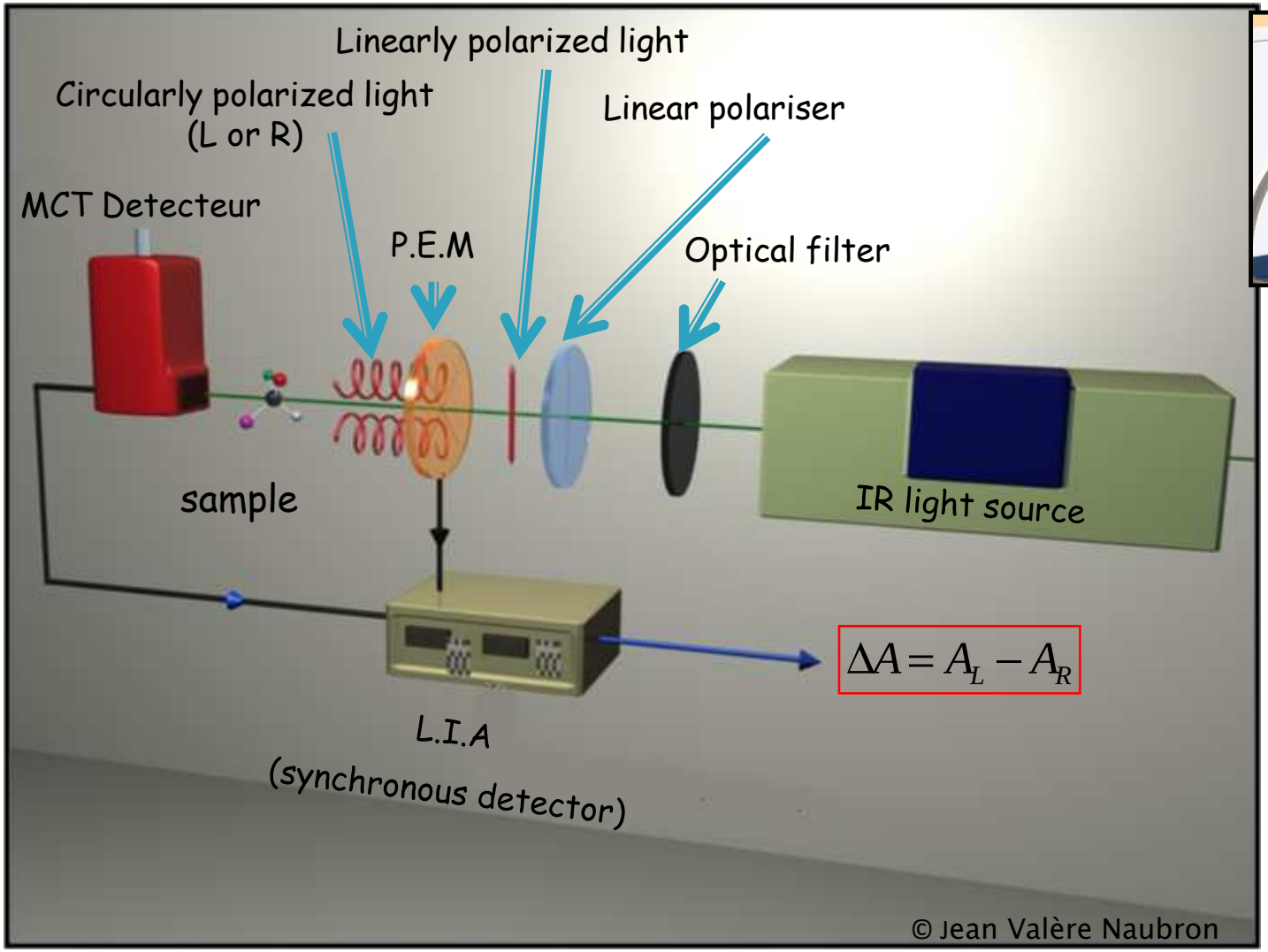
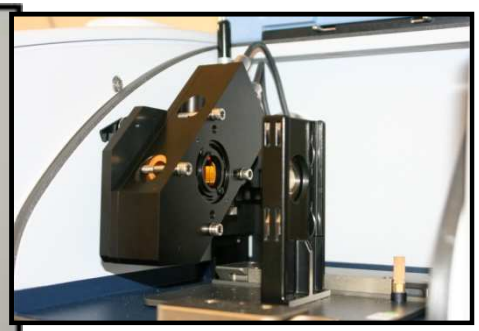



Fig 2. Circular dichroism effect of two light rays polarized

# VCD spectrometer



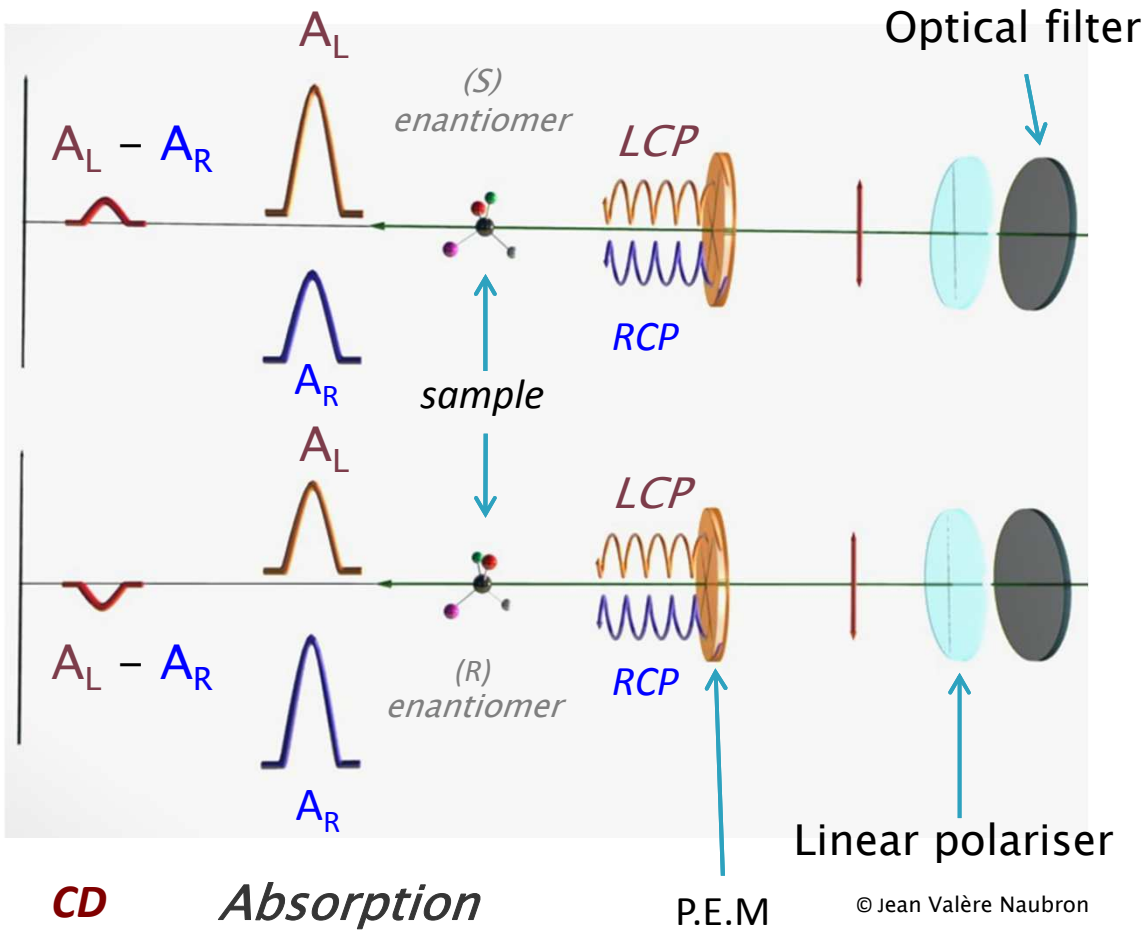
P.E.M

P.E.M: Photo Elastic Modulator

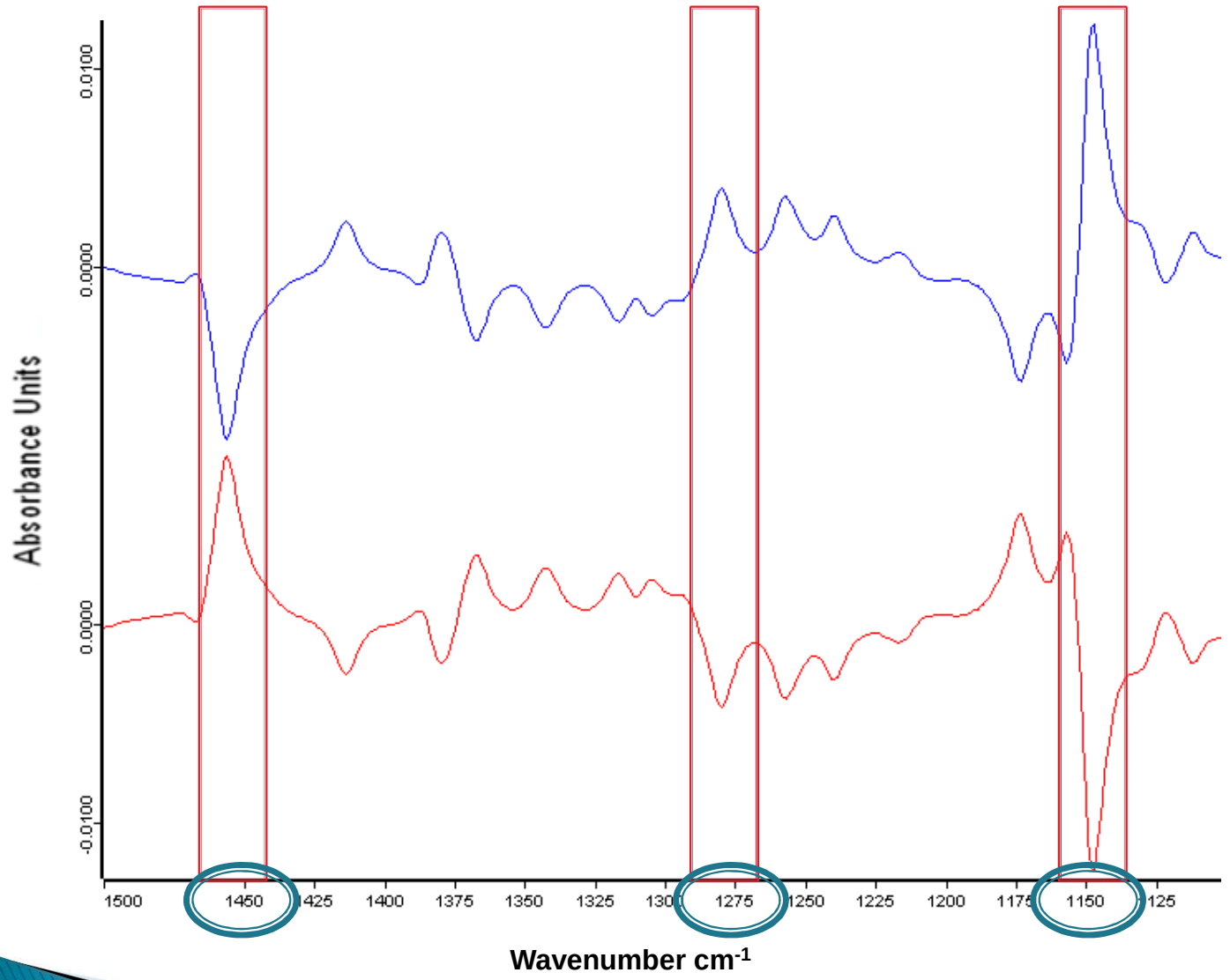
# VCD spectrometer

$$\Delta A = A_L - A_R$$

$$\frac{\Delta A}{A} \approx 10^{-4} \text{ à } 10^{-6} \text{ u.a}$$



# *VCD spectrometer*




(-)-β-thujone

(+)-β-thujone

Fig .3 VCD spectra of (+) and (-)-β-thujone

# *VCD spectrometer*


Fig 3. PMA50 (Bruker Optics)

**Spectroscopy parameters**:

- Spectral range : 1500-1100 $cm^{-1}$
- Accumulation :3h =>12000 scans
- Spectral resolution : 4 $cm^{-1}$

# *Materials & methods*

## Plant material

- Samples 1 and 2 of *Artemesia herba alba (AHA)* were collected respectively during April 2013 and July 2010 from Bechar in the south-west of Algeria and the Atlas Mountains, near Marrakech (Morocco).
- Sample 3 is AHA essential oil (EO) of Moroccan origin purchased from Naturosources.

*Fig 4. Artemesia herba alba*

# *Materials & methods*

## Extraction

Extraction was done in the laboratory of  Phytochemistry and Organic Synthesis (LPSO) in Algeria by steam distillation of water.

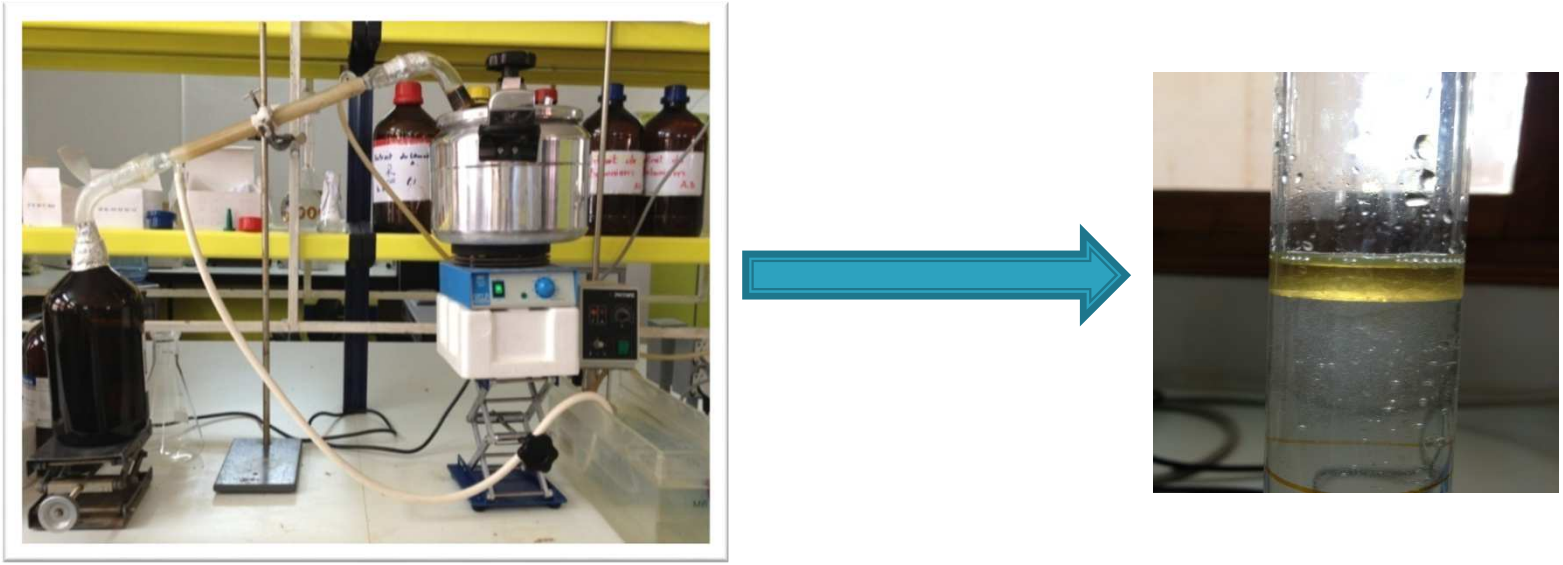


Fig 5. Extraction of *AHA* EO

# *Materials & methods*

## ❖Achiral Analysis

1- GC-MS
(IUT-Marseille)

→ Qualitative analysis: Compounds identification

Fig 6. Agilent Technologies GC 7890A with MS 5975C VL MSD

- Capillary column : HP-5MS.

- Column temperature program : 2 min at 80°C, gradient from 80 to 200°C at the rate of 5°C/min, 200 to 260°C at the rate of 20°C, and held at final temperature for 5 min.

- Carrier gas, helium (flow rate of 1.2 ml/min).

# *Materials & methods*

## ❖Achiral Analysis

2- GC-FID (LISA/METICA) → Quantitative analysis : relative %

Fig 7. Agilent Technologies GC 7890A

- Capillary column : HP-5.

- Column temperature program : 2 min at 80°C, gradient from 80 to 200°C at the rate of 5°C/min, 200 to 260°C at the rate of 20°C, and held at final temperature for 5 min.

- Carrier gas, $H_2$ (flow rate of 1.2 ml/min).

# *Materials & methods*

❖**Chiral analysis**

3- Chiral HPLC (CNRS, CHIROSCIENCES)

Fig 8. Lachrom-Elite

- Merck-Hitachi equipment able to test 12 chiral columns by varying also the mobile phase composition

- The detection is achieved by UV and a serial chirality detector: polarimeter or circular dichroism

Optimized conditions:

- Column : TCI-MBS.
- Carrier solvents : Heptane/Isopropanol 99/1, 1 ml/min.
- Detector: polarimeter

# Results

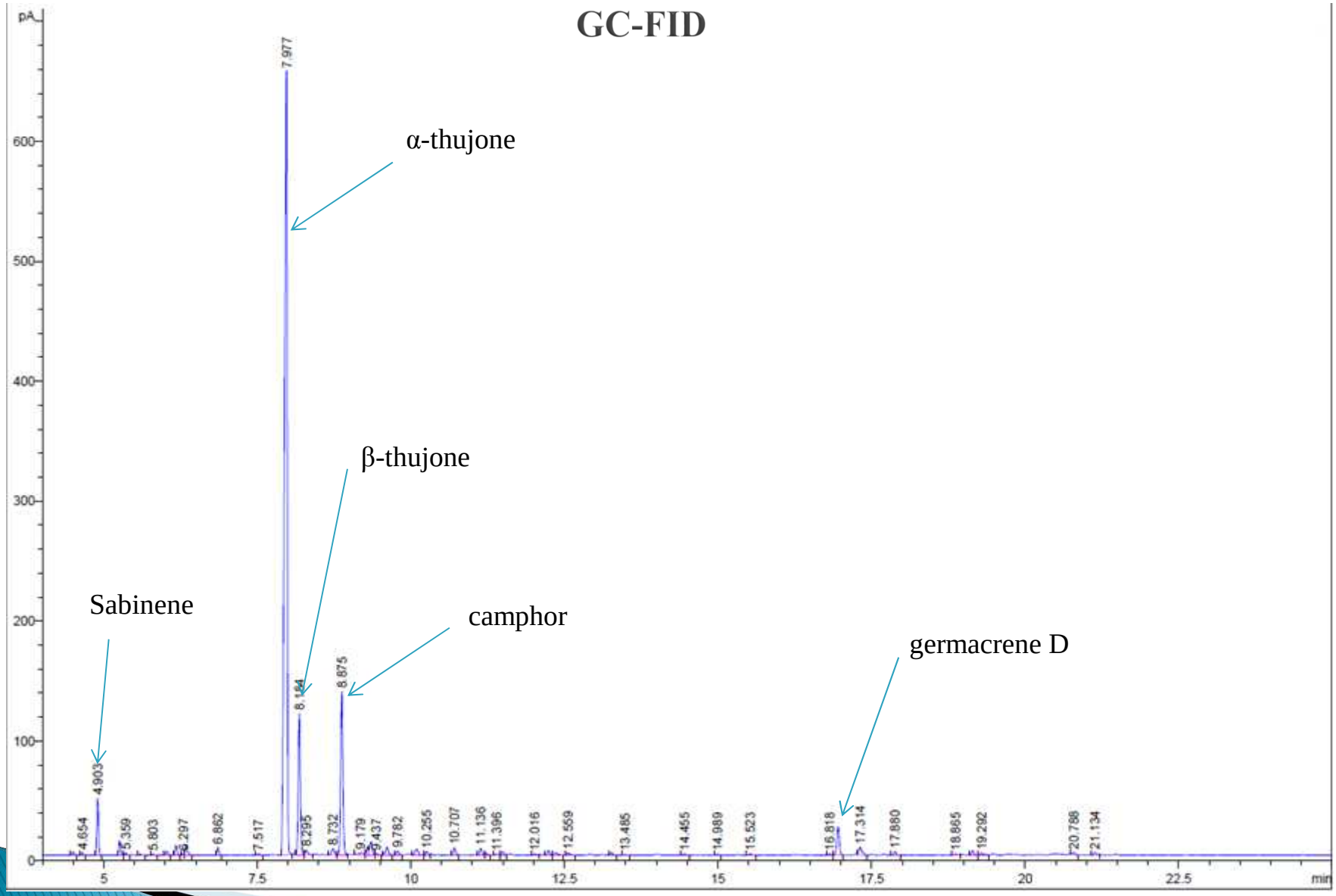



Fig 10. chromatogram of EO 1

# Results

## GC-MS & GC-FID

Table 1. Chemical composition of *AHA* EOs.

| RI | Major compounds | EO 1 % | EO 2 % | EO 3 % |
|---|---|---|---|---|
| 945 | Camphene | 3.55 | 3.22 | 6.96 |
| 974 | Sabinene | 0.93 | 1.13 | 0.96 |
| 1035 | Eucalyptol | 0.95 | 5.63 | 7.76 |
| 1107 | α-Thujone | 57.4 | 35.4 | 15.3 |
| 1115 | β-Thujone | 9.4 | 25.6 | 10.7 |
| 1150 | Camphor | 12.2 | 17.0 | 34.5 |
| 1173 | Borneol | 1.13 | 1.52 | 1.36 |
| 1491 | Germacrene D | 2.67 | 0.26 | 0.36 |
| 1497 | Bicyclogermacrene | 0.95 | 0.13 | 0.27 |
| Total | | 98.25 | 99.71 | 94.41 |
| Total chiral molecules | | 93.82 | 94.40 | 82.27 |

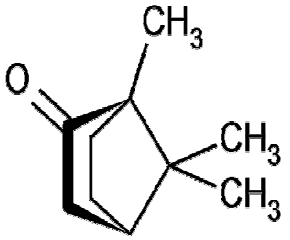
- EOs 1 and 2 are thujones chemotypes.
- EO 3 is camphor chemotype.
- These two chemotypes are founded in Algeria, Morroco, Tunisia, Egypt and Spain.

# Results

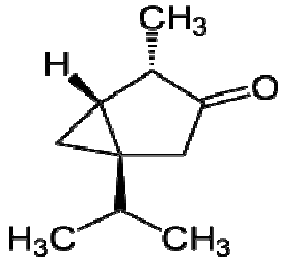
## Chiral HPLC

By chiral HPLC the sign of enantiomers is **(-)-α-thujone, (+)-β –thujone, (-)-camphor** and the enantiomeric excess is 100% for each compound.

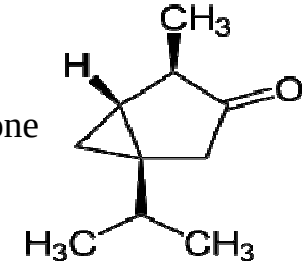
(-)-camphor




(-)-α-thujone




(+)-β-thujone



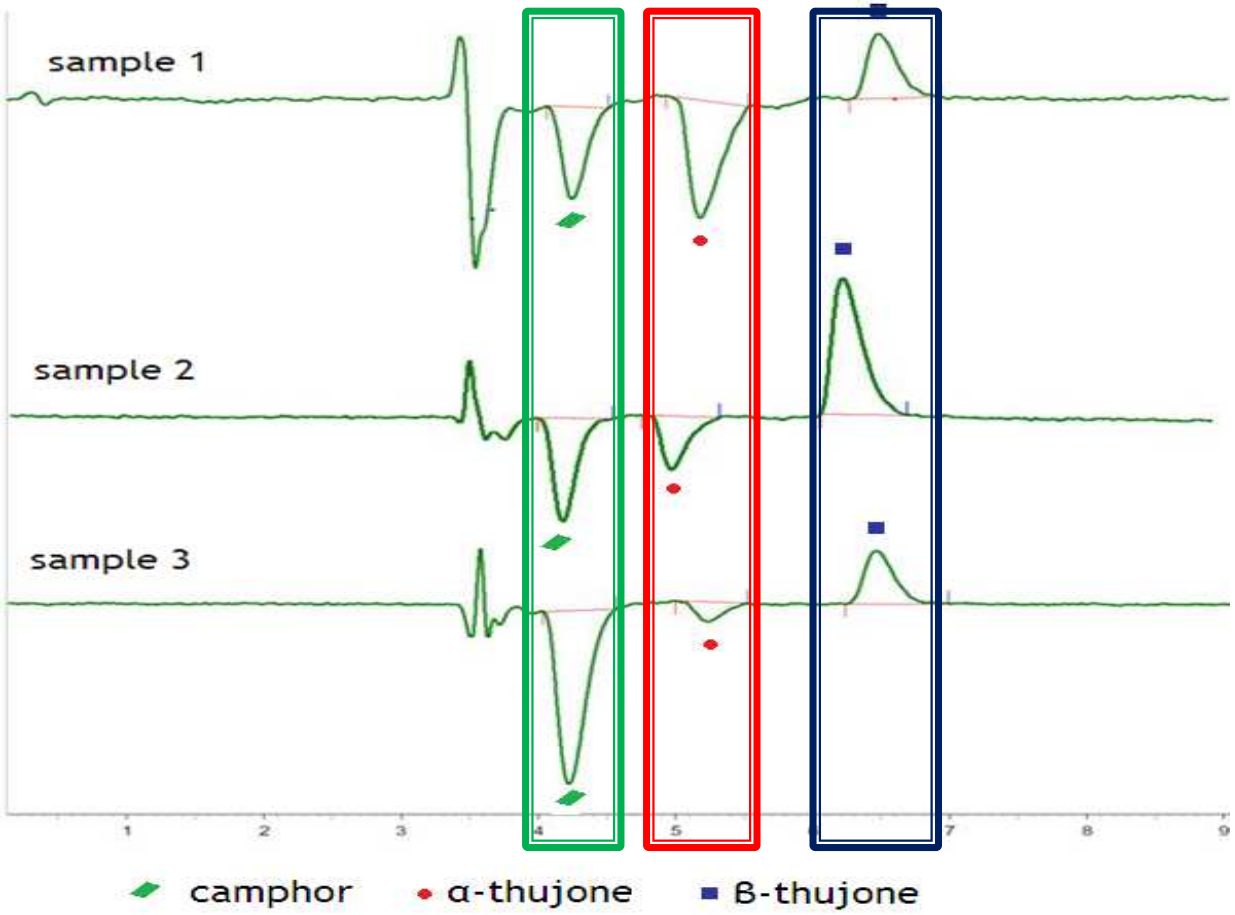




Fig 9. HPLC on TCI-MBS of crude EOs with polarimetric detection.

# *Results*

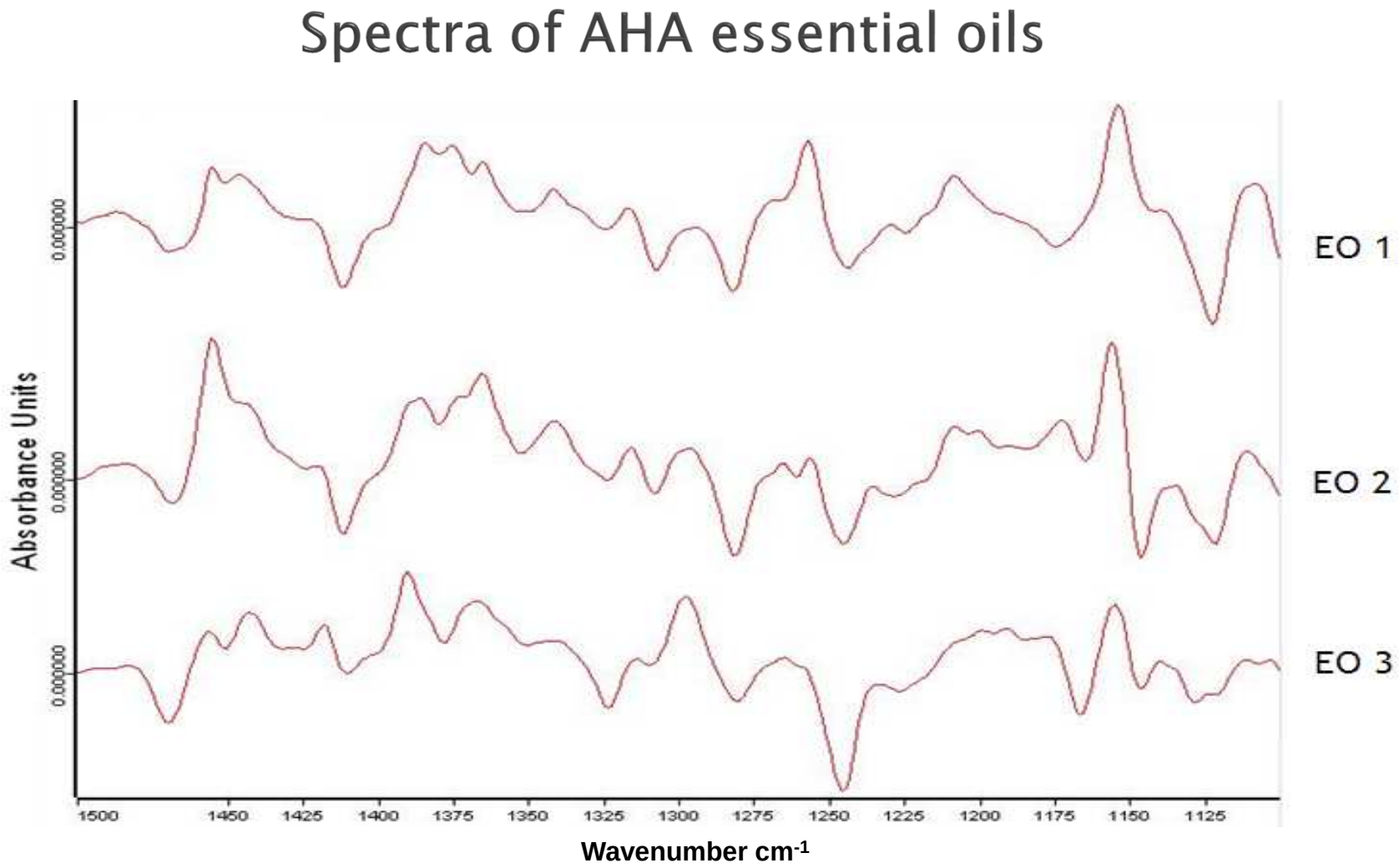



Fig 10. VCD spectra of EOs

# *Results*

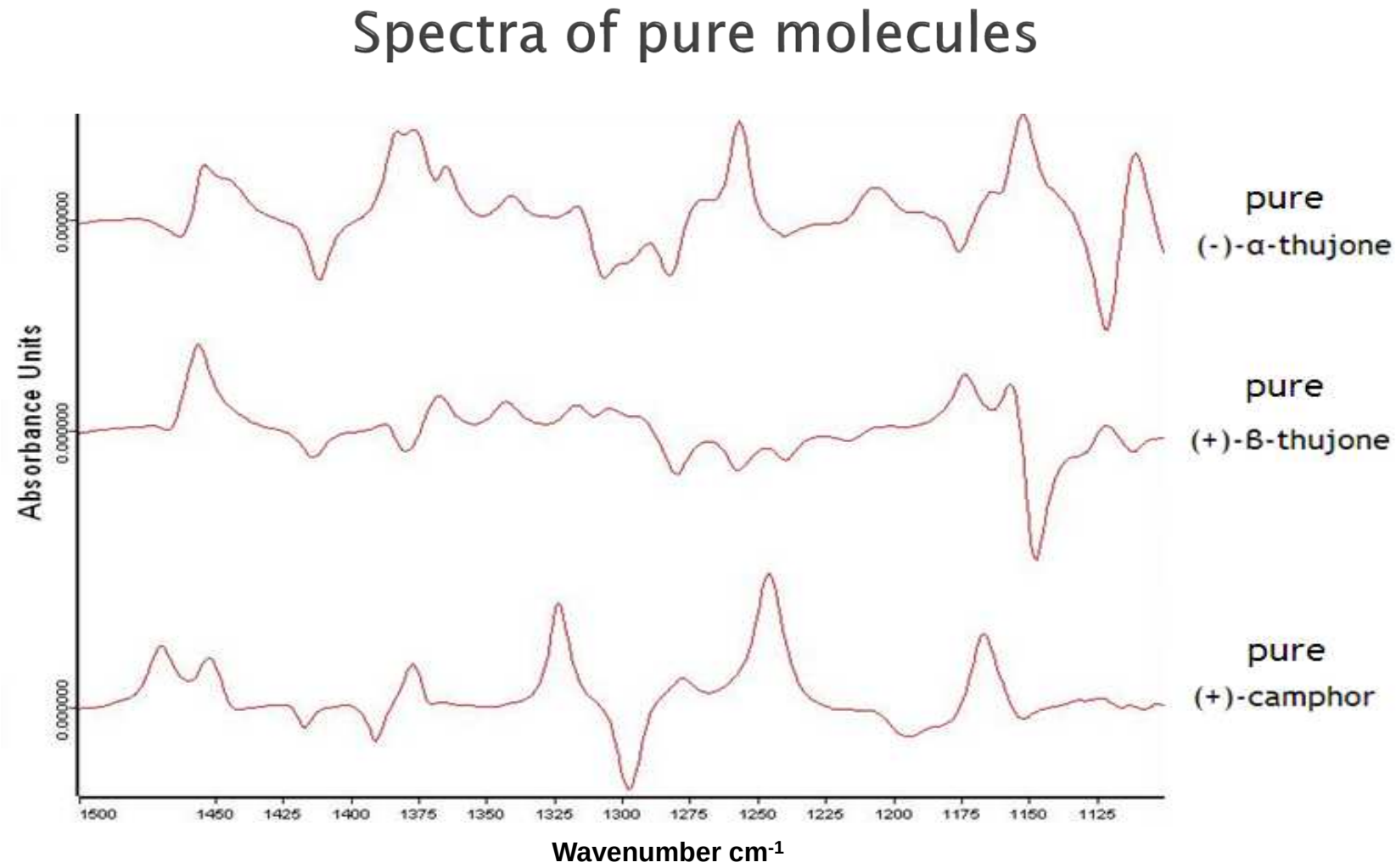
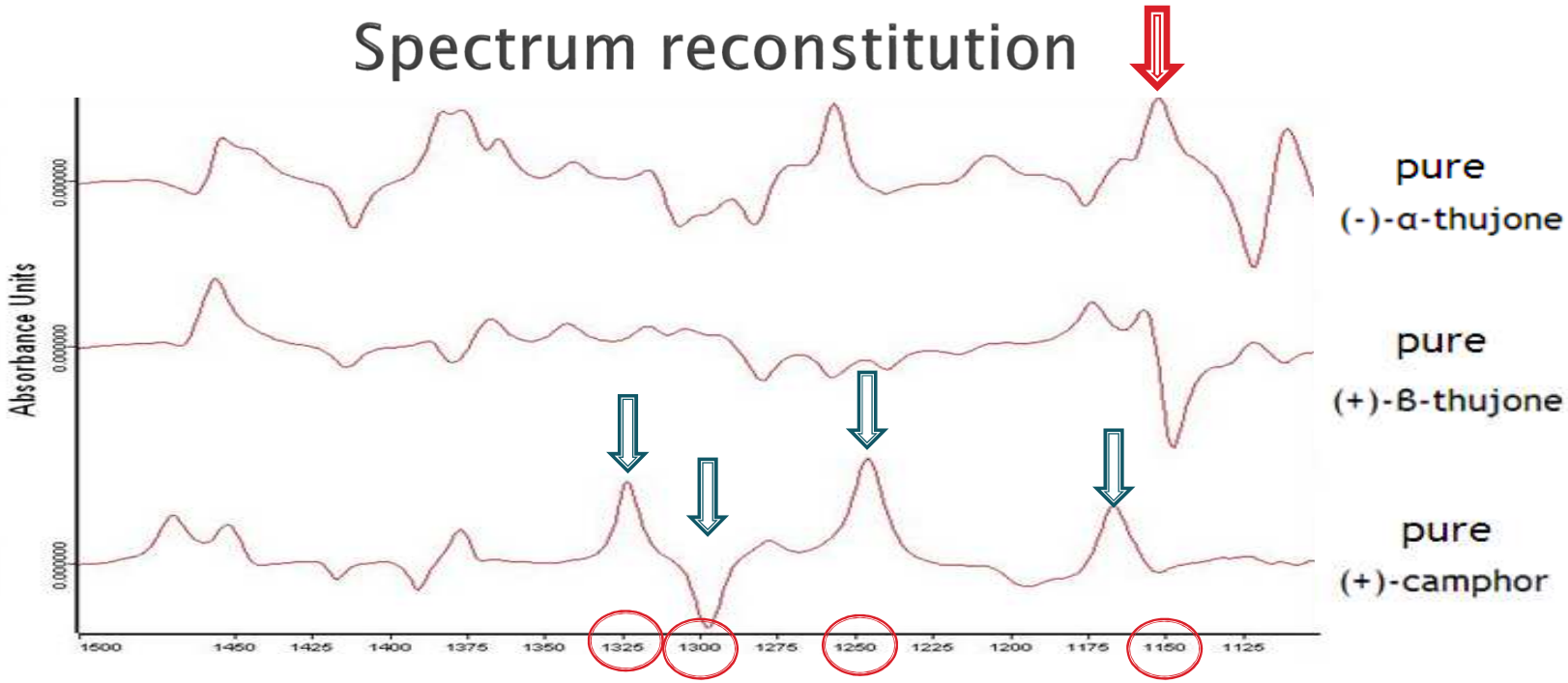
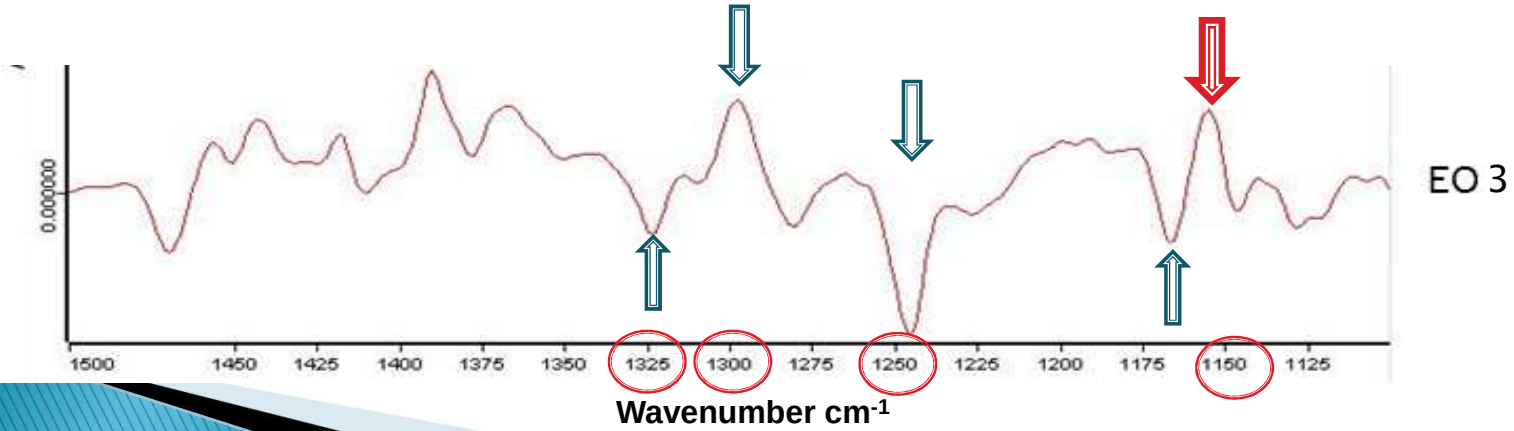



Fig 11.VCD spectra of major chiral compounds

# *Results*

## Spectrum reconstitution



# Results

## Spectrum reconstitution by Multiple Linear Regression (MLR)

MLR is a well-known statistical method based on ordinary least squares regression. The regression model can be written :

$$Y = \beta_0 + \beta_1 X_1 + ... + \beta_k X_k + e$$

Meaning that the observed response values are approximated by a linear combination of the values of the predictors. The coefficients of that combination are called regression coefficients or β –coefficients.

**X matrix**

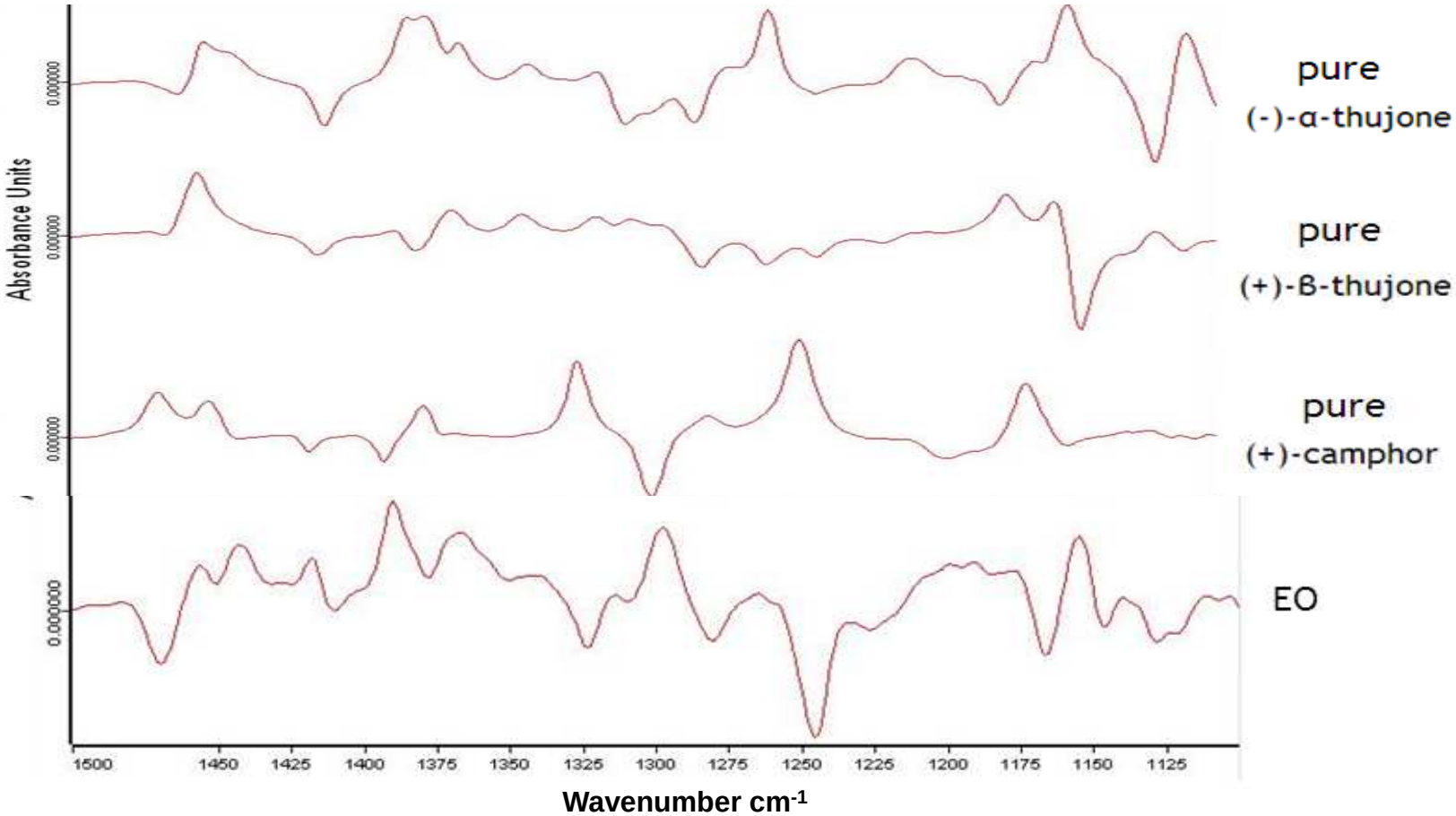
**Y matrix**

| Data Matrix(1 | | (-)-α-thujone | (+)-β-thujone | (+)-camphor | EO |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 1499,459 | 1 | -0,0001 | -0,0001 | 0,0000 | 0,0014 |
| 1498,495 | 2 | -0,0001 | -0,0001 | 0,0000 | 0,0013 |
| 1497,53 | 3 | -0,0001 | 0,0000 | 0,0000 | 0,0015 |
| 1496,566 | 4 | 0,0000 | 0,0000 | 0,0000 | 0,0018 |
| 1495,602 | 5 | 0,0000 | 0,0000 | 0,0001 | 0,0022 |
| 1494,637 | 6 | 0,0000 | 0,0001 | 0,0001 | 0,0025 |
| 1493,673 | 7 | 0,0000 | 0,0001 | 0,0001 | 0,0027 |
| 1492,709 | 8 | 0,0001 | 0,0002 | 0,0001 | 0,0028 |
| 1491,745 | 9 | 0,0001 | 0,0002 | 0,0001 | 0,0029 |
| 1490,78 | 10 | 0,0001 | 0,0002 | 0,0001 | 0,0030 |
| 1489,816 | 11 | 0,0001 | 0,0003 | 0,0002 | 0,0033 |
| 1488,852 | 12 | 0,0001 | 0,0003 | 0,0002 | 0,0036 |
| 1487,887 | 13 | 0,0001 | 0,0003 | 0,0003 | 0,0039 |
| 1486,923 | 14 | 0,0001 | 0,0003 | 0,0004 | 0,0040 |
| 1485,959 | 15 | 0,0001 | 0,0003 | 0,0004 | 0,0039 |
| 1484,995 | 16 | 0,0001 | 0,0004 | 0,0005 | 0,0037 |
| 1484,03 | 17 | 0,0001 | 0,0004 | 0,0005 | 0,0034 |
| 1483,066 | 18 | 0,0001 | 0,0004 | 0,0006 | 0,0030 |
| 1482,102 | 19 | 0,0001 | 0,0005 | 0,0006 | 0,0027 |
| 1481,137 | 20 | 0,0002 | 0,0005 | 0,0007 | 0,0023 |

# *Results*

## Spectrum reconstitution

$$\beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 = Y$$



# Results

## Spectrum reconstitution

Table 2. Predicted β-coefficients of VCD spectra.

| | | β-coefficients | | |
|---|---|---|---|---|
| β-coefficients | spectra | EO1 | EO2 | EO3 |
| $\beta_1$ | (-)-α-thujone | 0.73 | 0.39 | 0.27 |
| $\beta_2$ | (+)-β-thujone | 0.08 | 0.27 | 0.16 |
| $\beta_3$ | (+)-camphor | - 0.15 | -0.15 | -0.44 |

The MLR β-coefficient sign allows us to know the real sign of enantiomers (absolute configuration).

| spectra sign | | β sign | |
|---|---|---|---|
| - | **and** | + | **= α-thujone is (-)** |
| + | **and** | + | **= β -thujone is (+)** |
| + | **and** | - | **= Camphor is (-)** |

The results of the MLR prediction give us the same sign of enantiomers than chiral HPLC.

# Results

## Spectrum reconstitution

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| $\beta_2$ | (+)-β-thujone | 0.08 | 0.27 | 0.16 |
| $\beta_3$ | (+)-camphor | - 0.15 | -0.15 | -0.44 |

Table 3. Major compounds of *AHA* EOs.

| Major compounds | EO 1 (%) | EO 2 (%) | EO 3 (%) |
|---|---|---|---|
| (-)-α-thujone | 57.40 | 35.40 | 15.30 |
| (+)-β-thujone | 9.40 | 25.60 | 10.70 |
| (+)-camphor | 12.20 | 17.00 | 34.50 |

# Results

## Spectrum reconstitution

Table 2. Predicted β-coefficients of VCD spectra.

| | | β-coefficients | | |
|---|---|---|---|---|
| β-coefficients | spectra | EO1 (%) | EO2(%) | EO3(%) |
| $\beta_1$ | (-)-α-thujone | 73.14 | 39.51 | 27.53 |
| $\beta_2$ | (+)-β-thujone | 8.62 | 27.97 | 16.46 |
| $\beta_3$ | (+)-camphor | 15.05 | 15.04 | 44.37 |
| Total | | 96.81 | 82.52 | 88.36 |

Table 3. Major compounds of *AHA* EOs.

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|---|---|---|---|
| (-)-α-thujone | 57.40 | 35.40 | 15.30 |
| (+)-β-thujone | 9.40 | 25.60 | 10.70 |
| (+)-camphor | 12.20 | 17.00 | 34.50 |
| Total | 79 | 78 | 60.5 |

**Correction**

$$\%MLR = \frac{\beta i \times \sum \%CPG}{\sum \beta i}$$

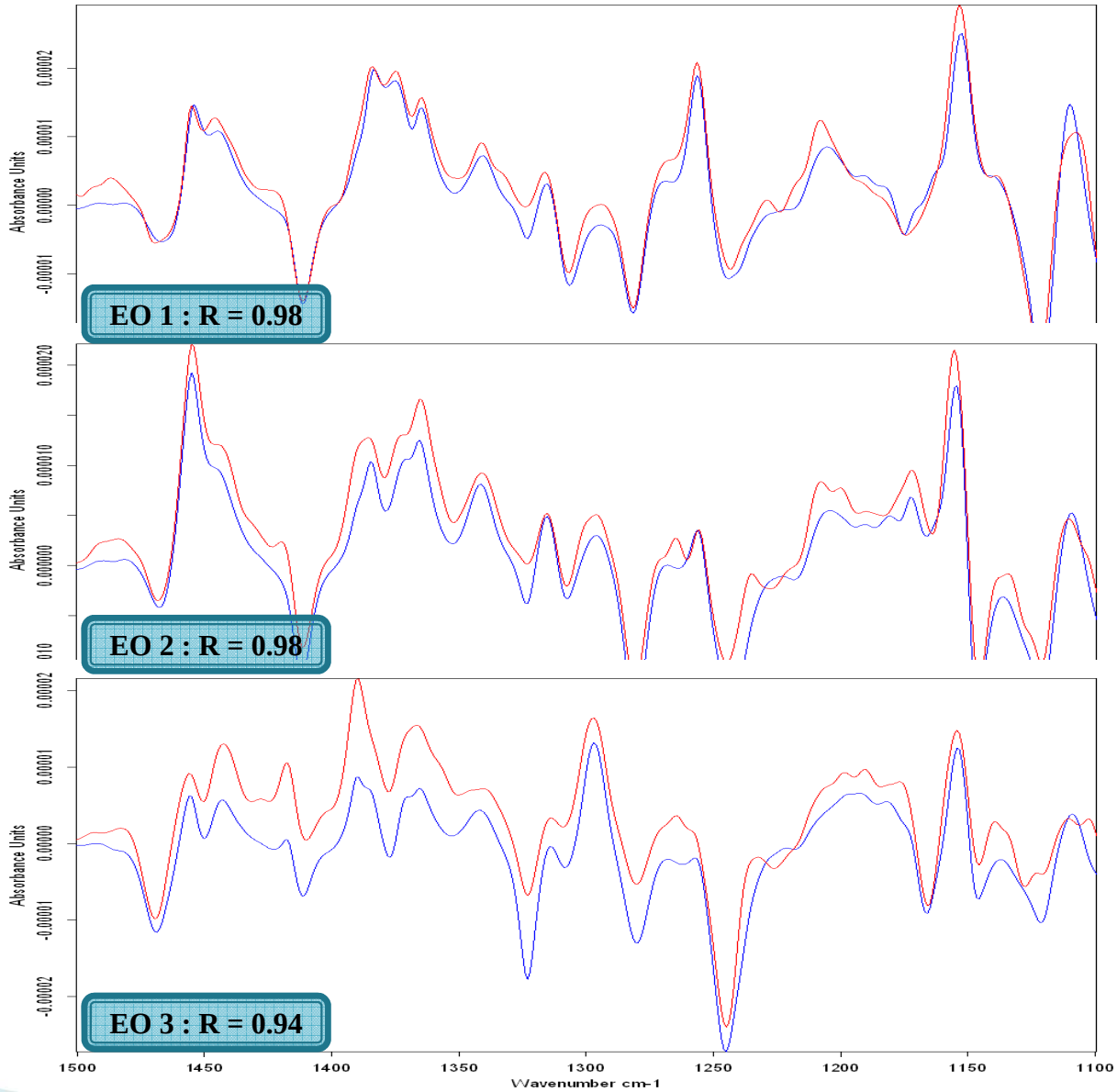
Table 4. % GC-FID & % MLR of EO1

| | EO 1 % | | EO 2 % | | EO 3 % | |
|---|---|---|---|---|---|---|
| Major compounds | % GC-FID | % MLR | % GC-FID | % MLR | % GC-FID | % MLR |
| α-thujone | 57.40 | 59,68 | 35.40 | 37,34 | 15.30 | 18,84 |
| β-thujone | 9.40 | 7,03 | 25.60 | 26,43 | 10.70 | 11,27 |
| camphor | 12.20 | 12,28 | 17.00 | 14,21 | 34.50 | 30,38 |

# Results

## Spectrum reconstitution

The modelized spectra of samples **1** and **2** have 98 % of similarity with measured spectra and sample **3**, 94 % of similarity.




modelized spectra

measured spectra

**R** : Pearson similarity coefficient

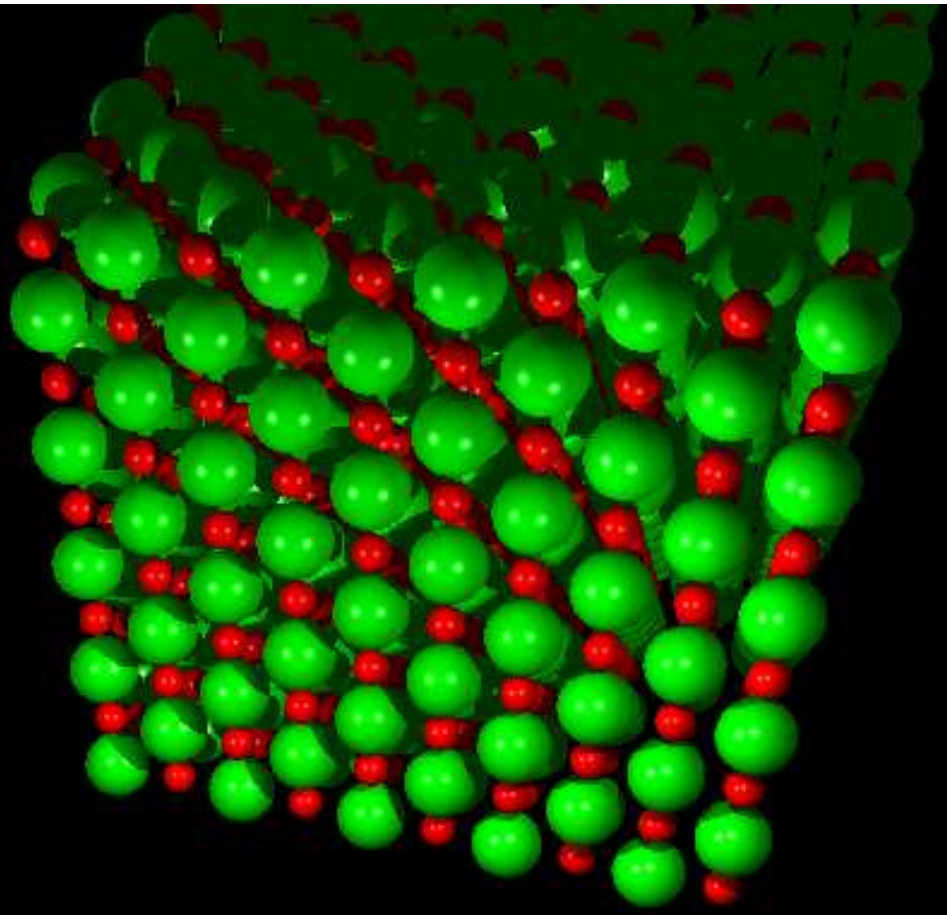
Fig 10. modelized & measured VCD spectra of EOs

# *Conclusion*

- A method based on the VCD spectra and Multi Linear Regression (MLR) to model the VCD spectra of *AHA* EOs.

- Determination of the absolute configuration of the major chiral constituents of *AHA* EOs

- Absolute configuration can be determined without using classic chiral chromatographic techniques.



*Merci de votre attention*