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Raman spectroscopy for identification and quantification analysis of essential oil varieties: a multivariate approach applied to lavender and lavandin essential oils

Sofia Lafhal,* Pierre Vanloot, Isabelle Bombarda, Robert Valls, Jacky Kister and Nathalie Dupuy

Lavender (*Lavandula angustifolia*) is used for cosmetics, perfumes and medicine (antimicrobial activity and relaxant properties) while lavandin (sterile hybrid of *L. angustifolia* P. Mill. \times *Lavandula latifolia* (L.f.) Medikus) is used for air fresheners, deodorants and soaps. These plants are widely cultivated for essential oil production. In this study, 104 samples were analyzed including 62 lavandin and 42 lavender oil samples from several varieties. The Raman spectra are similar but can be differentiated by chemometrics treatment. Data structure may be studied by PCA. A PLS regression model was used for quantitative analysis of the main compounds such as linalyl acetate, linalool and eucalyptol. The reference data were obtained by gas chromatography. The performance of the method was also tested to discriminate between the two species and the seven varieties (Abrial, Fine, Grosso, Maillette, Matherone, Sumian and Super) by PLS-DA regression. The examination of PLS and PLS-DA regression coefficients allowed for the identification of species and of the varieties' metabolomic markers. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: metabolomics; chemometrics; lavender; Raman; essential oil

Introduction

Lavender (*Lavandula angustifolia* Mill.) and lavandin (sterile hybrid of *L. angustifolia* P. Mill. \times *Lavandula latifolia* (L.f.) Medikus) belong to the Lamiaceae family and are classified into several species and varieties. The plant is native from the Mediterranean area and is widely cultivated for essential oil production and used in perfumes and cosmetic materials because of its pleasant smell and antimicrobial activity. This species has also been largely used as an ornamental plant.^[1,2] Among the main lavandin varieties (Grosso, Abrial, Sumian and Super), the Grosso variety is the most famous for its essential oil yield. Among the main lavender varieties (Fine, Maillette and Matherone), the Fine variety is the most famous for the same reason; the Matherone variety is currently less cultivated. Because of their different genetic background, the essential oils of lavender and lavandin varieties show different chemical profiles. If the differentiation of these species and varieties of essential oils can be achieved using gas chromatography^[3–5] and sniffing by specialists or gas chromatography—sniffing,^[6] the determination based on short time spectroscopic methods should be useful for essential oils classification. Molecular spectroscopy techniques such as mid-infrared (mid IR) or near infrared (NIR) have been shown to be useful for quality control of olive oils,^[7,8] rapid evaluation of essential oils such as thyme, oregano and chamomile^[9] and measurement of adulteration of virgin olive oils.^[10,11] However, the peaks in the NIR region are broad and weak, because they are combinations and overtones of the sample functional groups. For qualitative analysis, the mid IR spectroscopy has more applications, because the absorbance of functional groups can be displayed narrowly and intensely in the mid IR region. Fourier transform

infrared (FTIR) spectroscopy with attenuated total reflectance (ATR) or transmission cell accessories has been used to authenticate, identify or classify fats, fatty oils or essential oils.^[9,12,13] Raman spectroscopy is still used very sparingly in the food field, but it has advantages, such as its high sensitivity to C=C bonds and low sensitivity to water.^[14] Two instrumental methods can be employed with Raman spectroscopy: dispersive Raman spectroscopy (DRS) with a powerful laser in visible range, and Fourier transform Raman spectroscopy (FTRS). In FTRS, an NIR laser with less power is used, which requires the use of an interferometer. However, two studies on the detection of the adulteration of virgin olive oils have demonstrated the potential capabilities of DRS with 514.5-nm laser excitation^[15] and of a portable Raman spectrometer equipped with a diode laser (785 nm).^[16] Thus, portable Raman spectroscopy has been used to analyze vegetable and essential oils. The authors concluded that it was possible to perform quality control of essential oils even if the differentiation of varieties was not investigated.^[17] A dispersive Raman analysis showed a characterization of rosewood oil extracted from several parts of the Amazon tree *Aniba rosaeodora*, wood and leaf/branch oils were differentiated by PLS-DA regression.^[18] In this paper, the authors have shown examples of applications using Raman and IR vibrational spectroscopies for the evaluation of eucalyptus essential oil.^[19] In several current

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studies, analytical data have been treated by chemometrical methods such as principal component analysis (PCA),^[20,21] soft independent modeling of class analogies (SIMCA)^[22] and partial least square (PLS) regression.^[23–25] To our knowledge, there is few works on the determination of terpenoid composition of lavender and lavandin EOs^[26] and no work on the search of origin with respect to varieties using dispersive Raman spectroscopy (DRS). The aim of this study was to develop a direct and rapid test method to quantify terpenoid compounds in essential oils by DRS spectroscopy associated to chemometrics treatment. The potential of Raman spectroscopy for discriminating between the two species and the seven varieties was also investigated. The identification of metabolomic markers of the varieties was achieved.

Materials and methods

Essential oil samples

One hundred and four samples were analyzed including 62 lavandin oil samples and 42 lavender oil samples of several varieties, harvested in 2012 and 2013 in various French collect areas (Unknown department (00), Alpes-de-Haute-Provence (04), Ardèche (07), Drôme (26) and Vaucluse (84)). Essential oils studies were obtained from *lavandula* species: [*L. angustifolia* Miller] (lavender) and its hybrid [*L. angustifolia* Miller × *L. latifolia* Linnaeus fil. Medikus] (lavandin) type France. Essential oil samples are divided into varieties including: Fine (FI, $n = 12$), Maillette (MA, $n = 18$) and Matherone (MT, $n = 12$) varieties for lavender samples and Abrial (AB, $n = 11$), Grosso (GR, $n = 22$), Sumian (SU, $n = 14$) and Super (SP, $n = 15$) varieties for lavandin samples.

Pure standard substances

Pure standard substances linalyl acetate, borneol, linalool, β -caryophyllene, eucalyptol and camphor were purchased from France lavande, Alpha aesar, Fluka, TCI, Merck and Alpha aesar, respectively.

Gas chromatography

GC-MS analysis

An Agilent Technologies GC instrument equipped with a GC 7890A gas chromatograph system, a MS 5975C VL MSD mass spectrometer detector and a HP-5MS capillary column J&W Scientific (30 m × 0.25 mm, 0.25 μ m film thickness) was used. The data acquisition and processing were performed using the MSD Chemstation E.01.01.335 (Agilent) software.

One microliter of diluted essential oil (80 μ l in 1.5 ml of ethanol) was injected. The experimental conditions were: solvent delay, 2 min; column temperature program, 2 min at 80 °C, then 80 °C to 200 °C (5 °C/min), then 200 °C to 260 °C (20 °C/min), and held at final temperature for 5 min; temperature injector (split ratio 60) and detector were 250 °C; carrier gas was helium at a flow rate of 1.2 ml/min; ionization voltage 70 eV; electron multiplier, 1 kV.

GC analysis

An Agilent Technologies gas chromatograph (7890A) was used with a flame ionization detector (FID) and a HP5 capillary column J&W Scientific (30 m × 0.25 mm, 0.25 μ m film thickness). The data acquisition and processing were performed using the Chemstation B.04.03-SP1 (87) (Agilent) software. Oven temperature was programmed 2 min at 80 °C, then 80 °C to 200 °C (5 °C/min), then

200 °C to 260 °C (20 °C/min), and held at final temperature for 5 min. Injector and detector temperatures were set at 250 °C. Hydrogen was the carrier gas at a flow rate of 1.2 ml/min. Linear retention indices were calculated with reference to *n*-alkanes (C8–C40).

Raman spectroscopy

Spectra were collected with an Almega (Thermo-fisher Scientific Nicolet) Raman spectrometer equipped with a Nd: YVO₄ diode-pumped solid-state (DPSS) laser (532 nm). The minimum and maximum powers at the output of the laser head were 15 and 150 mW, respectively. All spectra were taken in the 180° (backscattering) geometry. The detector was a charge coupled device (CCD). Spectra of the samples placed in a quartz cell (2 mm) were recorded with 2 cm^{−1} resolution and two accumulations of 15 s each at full laser power in the range 90–4000 cm^{−1} using the Omnic 7.2 software (Thermo-fisher Scientific Nicolet). The Raman spectrometer was situated in an air-conditioned room (21 °C).

Extended multivariate scatter correction (EMSC)

During the data processing, the EMSC pretreatment technique was used. In the EMSC pretreatment five correction factors were calculated for each spectrum by polynomial regression of the spectrum on an ideal spectrum, which was usually the mean spectrum of the calibration set.

The EMSC model was:

$$x_i = a_i + b_i x_{ref} + h_i k_i + d_i \bar{\nu} + e_i \bar{\nu}^2 + \varepsilon_i \quad (1)$$

where coefficient a_i represents the additive effect (baseline offset), b_i the multiplicative effect (path length), d_i and e_i the wavelength dependent light scatter effect, $h_i k_i$ the chemical effects, ε_i the vector containing residual spectrum, $\bar{\nu}$ the vector of wavenumbers and x_{ref} the mean spectrum of the calibration set.

These correction factors \hat{a}_i , \hat{b}_i , \hat{h}_i , \hat{d}_i and \hat{e}_i were estimated by least squares regression of each spectrum, and each spectrum was corrected by EMSC as follows:

$$x_i^* = \left(x_i - \hat{a}_i - \hat{h}_i k_i - \hat{d}_i - \hat{e}_i^2 \right) / \hat{b}_i \quad (2)$$

All EMSC pretreated spectra x_i^* constituted the matrix used to perform PCA and PLS regression.^[27]

Chemometrics analysis

Chemometrics analysis of the Raman spectra was performed using a commercial software program (The UNSCRAMBLER X V.10.3 from CAMO/Software, Oslo, Norway). Principal Component Analysis (PCA) was used such as an interpretable overview of the main information in a multidimensional array. PLS-DA regression was carried out using an exclusive binary coding scheme with one bit per class. For the codification of samples, the two species corresponding to the lavender and lavandin were arbitrarily classified in that order. For instance, a lavender sample was codified by the vector {1; 0}. The sample was then assigned to the class showing the highest membership value. In front of the difficulty of calibrating and predicting species with binary variables it was necessary to discriminate the results between the initial values 0 or 1. Samples values lower than 0.5 and higher than 1.5 were identified as outside the defined species, and samples with values between 0.5 and 1.5 were identified as belonging to the defined specie. The same protocol was used to predict varieties. The seven varieties corresponding

to AB, GR, SU, SP, FI, MA and MT were arbitrarily classified in that order. For instance, an AB sample was codified by the vector {1; 0; 0; 0; 0; 0; 0}. To build PLS regression models, 70 samples were used: (FI, $n=8$), (MA, $n=12$), (MT, $n=8$), (AB, $n=8$), (GR, $n=14$), (SU, $n=10$) and (SP, $n=10$) and the validation step was performed by full cross validation. To test the performance of the models, 34 samples were used: (FI, $n=4$), (MA, $n=6$), (MT, $n=4$), (AB, $n=3$), (GR, $n=8$), (SU, $n=4$) and (SP, $n=5$). The evaluation of errors in the calibration or prediction was carried out by computing the standard error of calibration or prediction (SEC and SEP). Another useful parameter was relative error of prediction (REP), which shows the predictive ability of the model. Finally, the evaluation of regression coefficient was carried out by computing the Pearson coefficient.

Results and discussion

Gas chromatography

The identification of the compounds was based on the comparison of their mass spectra with those of Wiley275 and NIST05a libraries as well as by comparing their retention indices with those of authentic samples. Tables 1 and 2 list the relative composition determined from peak areas (mean and range) for the 28 compounds accounting for more than 0.1% of the EOs for the lavender and lavandin varieties studied. Among the constituents investigated,

there were eight monoterpenes, three sesquiterpenes and seventeen oxygenated derivatives. *Trans*- β -ocimene (0.16–10.71%) was the major monoterpene, followed by limonene (<0.1–1.17%). β -Caryophyllene (1.03–7.07%) was the major sesquiterpene. Linalool (14.28–50.20%) and linalyl acetate (19.39–46.22%) were the main oxygenated constituents, followed by minor amounts of camphor (0.17–10.14%) and terpinen-4-ol (0.07–8.11%). A high content of eucalyptol (0.80–10.52%) was also identified, but it was coeluted with *cis*- β -ocimene in our experimental chromatographic conditions. As shown in Tables 1 and 2, there are significant differences between the two species particularly regarding camphor content. The low camphor content in lavender (0.43%) compared to lavandin (6.75%) justifies its use in the perfume industry and the use of lavandin in the toiletries industry. Other minor differences in compound contents were observed, showing a potential differentiation between lavender and lavandin EOs and between each species.

Raman spectroscopy

Figure 1 shows the characteristic Raman spectra of lavender and lavandin EOs in the 1800–400 cm^{-1} range. Even though the compositions of lavender and lavandin essential oils differ greatly, their spectra are very similar. The spectra of the six pure terpenoids present in higher amount in lavender and lavandin EOs were recorded (Fig. 2). The assignment of major bands was carried out

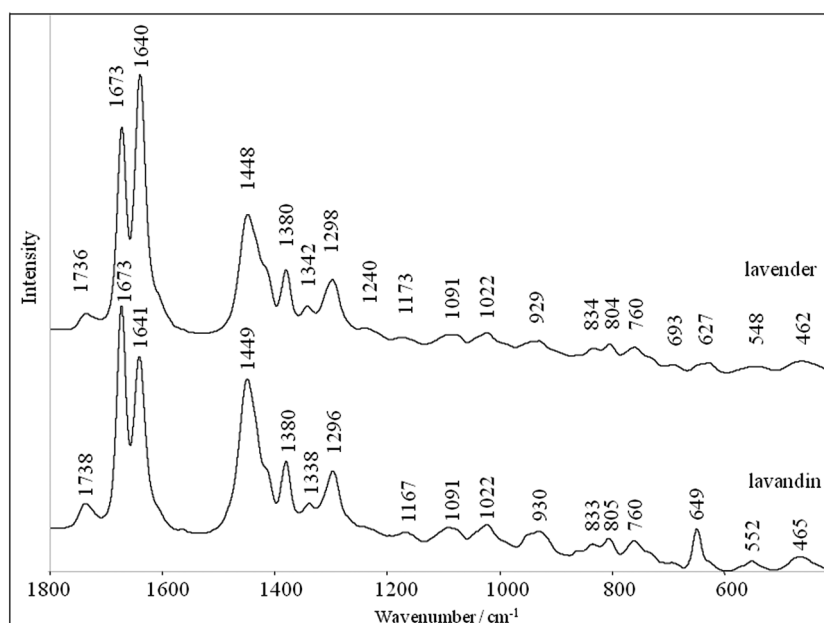
Table 1. Chemical composition of lavender EO varieties

| RI ^a | Compounds | FI ($n=15$) | | | MA ($n=20$) | | | MT ($n=13$) | | | Lavender ($n=53$) | | |
|-----------------|--|---------------|-------|-------|---------------|-------|-------|---------------|-------|-------|---------------------|-------|-------|
| | | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean |
| 923 | α -Pinene | 0.09 | 0.33 | 0.16 | 0.00 | 0.17 | 0.08 | 0.00 | 0.21 | 0.08 | 0.00 | 0.33 | 0.11 |
| 944 | Camphene | 0.00 | 0.20 | 0.11 | 0.14 | 0.34 | 0.21 | 0.00 | 0.11 | 0.08 | 0.00 | 0.34 | 0.14 |
| 973 | Sabinene | 0.12 | 0.57 | 0.32 | 0.15 | 0.62 | 0.38 | 0.15 | 0.61 | 0.30 | 0.12 | 0.62 | 0.34 |
| 980 | β -Pinene | 0.00 | 0.31 | 0.09 | 0.00 | 0.14 | 0.04 | 0.00 | 0.08 | 0.02 | 0.00 | 0.31 | 0.05 |
| 985 | 3-Octanone | 0.30 | 1.27 | 0.67 | 0.75 | 1.64 | 1.24 | 0.13 | 0.74 | 0.36 | 0.13 | 1.64 | 0.83 |
| 989 | β -Myrcene | 0.28 | 0.85 | 0.42 | 0.15 | 0.64 | 0.34 | 0.25 | 0.66 | 0.44 | 0.15 | 0.85 | 0.40 |
| 1012 | Hexyl acetate | 0.15 | 0.41 | 0.26 | 0.18 | 0.60 | 0.36 | 0.00 | 0.37 | 0.11 | 0.00 | 0.60 | 0.26 |
| 1030 | Limonene | 0.17 | 0.66 | 0.32 | 0.06 | 0.33 | 0.15 | 0.00 | 0.32 | 0.12 | 0.00 | 0.66 | 0.20 |
| 1034 | Eucalyptol and <i>cis</i> - β -ocimene | 2.69 | 5.88 | 4.19 | 0.80 | 2.92 | 1.36 | 3.89 | 8.43 | 6.07 | 0.80 | 8.43 | 3.52 |
| 1044 | <i>trans</i> - β -ocimene | 2.18 | 4.69 | 3.02 | 0.38 | 1.99 | 0.91 | 5.09 | 10.71 | 7.27 | 0.38 | 10.71 | 3.29 |
| 1073 | Linalool oxide | 0.11 | 0.24 | 0.17 | 0.13 | 0.54 | 0.40 | 0.07 | 0.20 | 0.12 | 0.07 | 0.54 | 0.25 |
| 1087 | α -Terpinolene | 0.00 | 0.23 | 0.10 | 0.00 | 0.50 | 0.17 | 0.00 | 0.20 | 0.07 | 0.00 | 0.50 | 0.12 |
| 1098 | Linalool | 22.68 | 31.16 | 27.51 | 13.16 | 50.20 | 39.78 | 14.28 | 25.40 | 20.48 | 13.16 | 50.20 | 30.72 |
| 1106 | Octen-1-ol acetate | 0.46 | 1.25 | 0.90 | 0.39 | 0.93 | 0.58 | 0.71 | 1.37 | 0.94 | 0.39 | 1.37 | 0.78 |
| 1145 | Hexyl isobutyrate | 0.00 | 0.17 | 0.08 | 0.00 | 0.10 | 0.06 | 0.00 | 0.10 | 0.03 | 0.00 | 0.17 | 0.06 |
| 1150 | Camphor | 0.19 | 1.04 | 0.36 | 0.39 | 0.91 | 0.58 | 0.17 | 0.43 | 0.28 | 0.17 | 1.04 | 0.43 |
| 1168 | Lavandulol | 0.48 | 2.13 | 0.97 | 0.00 | 3.36 | 0.33 | 0.48 | 4.28 | 1.54 | 0.00 | 4.28 | 0.86 |
| 1171 | Borneol | 0.75 | 1.50 | 1.13 | 1.20 | 2.39 | 1.75 | 0.58 | 1.32 | 0.88 | 0.58 | 2.39 | 1.32 |
| 1183 | Terpinen-4-ol | 2.10 | 8.11 | 3.99 | 0.07 | 18.18 | 1.22 | 1.15 | 2.54 | 1.79 | 0.07 | 18.18 | 2.24 |
| 1193 | Hexyl butyrate | 0.26 | 0.47 | 0.36 | 0.38 | 0.73 | 0.56 | 0.00 | 0.40 | 0.14 | 0.00 | 0.73 | 0.38 |
| 1197 | α -Terpineol | 0.37 | 1.47 | 0.76 | 0.09 | 1.10 | 0.72 | 0.18 | 1.37 | 0.83 | 0.09 | 1.47 | 0.76 |
| 1260 | Linalyl acetate | 34.66 | 43.18 | 37.98 | 31.63 | 46.22 | 38.04 | 36.29 | 45.79 | 39.69 | 31.63 | 46.22 | 38.47 |
| 1293 | Lavandulyl acetate | 2.58 | 7.35 | 4.34 | 0.31 | 7.81 | 1.06 | 2.77 | 7.06 | 5.17 | 0.31 | 7.81 | 3.20 |
| 1386 | Geranyl acetate | 0.20 | 0.86 | 0.54 | 0.08 | 0.79 | 0.54 | 0.13 | 0.87 | 0.49 | 0.08 | 0.87 | 0.53 |
| 1429 | β -Caryophyllene | 4.11 | 6.11 | 5.11 | 2.69 | 7.51 | 3.82 | 4.92 | 7.07 | 5.99 | 2.69 | 7.51 | 4.81 |
| 1460 | β -Farnesene | 0.49 | 2.50 | 1.79 | 1.08 | 2.35 | 1.83 | 2.01 | 3.56 | 2.87 | 0.49 | 3.56 | 2.10 |
| 1492 | Germacren D | 0.44 | 1.09 | 0.77 | 0.14 | 0.55 | 0.27 | 0.47 | 0.91 | 0.68 | 0.14 | 1.09 | 0.54 |
| 1594 | Caryophyllene oxide | 0.29 | 0.79 | 0.52 | 0.29 | 0.94 | 0.48 | 0.25 | 0.82 | 0.46 | 0.25 | 0.94 | 0.49 |

^aRI: Retention indices on HP-5 capillary column.

Table 2. Chemical composition of lavandin EO varieties

| RI ^a | Compounds | AB (n = 11) | | | GR (n = 22) | | | SP (n = 16) | | | SU (n = 14) | | | Lavandin (n = 63) | | |
|-----------------|--|-------------|-------|-------|-------------|-------|-------|-------------|-------|-------|-------------|-------|-------|-------------------|-------|-------|
| | | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean |
| 923 | α -Pinene | 0.21 | 0.49 | 0.32 | 0.22 | 0.54 | 0.36 | 0.07 | 0.16 | 0.12 | 0.06 | 0.47 | 0.23 | 0.06 | 0.54 | 0.26 |
| 944 | Camphene | 0.19 | 0.38 | 0.27 | 0.17 | 0.33 | 0.24 | 0.1 | 0.19 | 0.14 | 0.06 | 0.35 | 0.20 | 0.06 | 0.38 | 0.22 |
| 973 | Sabinene | 0.33 | 0.77 | 0.54 | 0.18 | 0.56 | 0.32 | 0.00 | 0.18 | 0.11 | 0.13 | 0.46 | 0.29 | 0.00 | 0.77 | 0.30 |
| 980 | β -Pinene | 0.24 | 0.59 | 0.36 | 0.20 | 0.51 | 0.33 | 0.00 | 0.11 | 0.05 | 0.00 | 0.57 | 0.18 | 0.00 | 0.59 | 0.24 |
| 985 | 3-Octanone | 0.11 | 0.33 | 0.19 | 0.00 | 0.00 | 0.00 | 0.37 | 0.91 | 0.61 | 0.11 | 1.28 | 0.71 | 0.00 | 1.28 | 0.35 |
| 989 | β -Myrcene | 0.32 | 0.53 | 0.43 | 0.39 | 0.68 | 0.52 | 0.31 | 0.83 | 0.54 | 0.28 | 0.58 | 0.43 | 0.28 | 0.83 | 0.49 |
| 1012 | Hexyl acetate | 0.09 | 0.25 | 0.17 | 0.08 | 0.22 | 0.14 | 0.25 | 0.69 | 0.44 | 0.00 | 0.19 | 0.07 | 0.00 | 0.69 | 0.20 |
| 1030 | Limonene | 0.57 | 0.86 | 0.68 | 0.52 | 0.84 | 0.66 | 0.00 | 1.08 | 0.68 | 0.40 | 1.18 | 0.80 | 0.00 | 1.18 | 0.72 |
| 1034 | Eucalyptol and <i>cis</i> - β -ocimene | 7.29 | 10.46 | 8.44 | 4.35 | 7.66 | 5.34 | 2.95 | 4.30 | 3.59 | 2.79 | 10.52 | 7.01 | 2.79 | 10.52 | 5.96 |
| 1044 | <i>trans</i> - β -ocimene | 2.25 | 4.16 | 2.91 | 0.16 | 0.45 | 0.30 | 1.11 | 1.91 | 1.55 | 0.42 | 3.11 | 1.62 | 0.16 | 4.16 | 1.36 |
| 1073 | Linalool oxide | 0.10 | 0.17 | 0.13 | 0.09 | 0.18 | 0.12 | 0.07 | 0.15 | 0.10 | 0.11 | 0.16 | 0.13 | 0.07 | 0.18 | 0.12 |
| 1087 | α -Terpinolene | 0.31 | 0.42 | 0.36 | 0.29 | 0.41 | 0.36 | 0.20 | 0.34 | 0.25 | 0.23 | 0.41 | 0.32 | 0.20 | 0.42 | 0.33 |
| 1098 | Linalool | 30.67 | 37.25 | 34.48 | 28.44 | 37.09 | 33.36 | 30.07 | 39.31 | 34.90 | 35.40 | 46.18 | 41.00 | 28.44 | 46.18 | 35.87 |
| 1106 | Octen-1-ol acetate | 0.32 | 0.55 | 0.43 | 0.22 | 0.49 | 0.30 | 0.07 | 0.42 | 0.26 | 0.12 | 0.31 | 0.23 | 0.07 | 0.55 | 0.29 |
| 1145 | Hexyl isobutyrate | 0.16 | 0.20 | 0.18 | 0.15 | 0.25 | 0.19 | 0.10 | 0.18 | 0.14 | 0.14 | 0.23 | 0.18 | 0.10 | 0.25 | 0.18 |
| 1150 | Camphor | 8.23 | 10.14 | 9.30 | 6.15 | 8.53 | 7.05 | 4.04 | 5.68 | 4.79 | 4.82 | 9.03 | 6.26 | 4.04 | 10.14 | 6.75 |
| 1168 | Lavandulol | 0.36 | 1.00 | 0.70 | 0.34 | 1.16 | 0.59 | 0.10 | 0.66 | 0.32 | 0.00 | 1.31 | 0.36 | 0.00 | 1.31 | 0.47 |
| 1171 | Borneol | 1.94 | 3.55 | 2.83 | 2.31 | 4.00 | 2.98 | 2.09 | 3.41 | 2.82 | 1.97 | 8.48 | 5.34 | 1.94 | 8.48 | 3.55 |
| 1183 | Terpinen-4-ol | 0.48 | 1.49 | 0.95 | 1.86 | 4.93 | 3.41 | 0.09 | 0.86 | 0.25 | 0.15 | 3.59 | 1.01 | 0.09 | 4.93 | 1.62 |
| 1193 | Hexyl butyrate | 0.32 | 0.55 | 0.40 | 0.31 | 0.55 | 0.42 | 0.43 | 0.86 | 0.69 | 0.31 | 0.71 | 0.49 | 0.31 | 0.86 | 0.50 |
| 1197 | α -Terpineol | 0.48 | 0.97 | 0.76 | 0.37 | 1.65 | 0.90 | 0.26 | 1.86 | 0.99 | 0.65 | 1.23 | 0.95 | 0.26 | 1.86 | 0.91 |
| 1260 | Linalyl acetate | 22.80 | 28.33 | 25.39 | 27.94 | 36.52 | 31.28 | 34.71 | 44.09 | 38.53 | 19.40 | 29.72 | 23.82 | 19.40 | 44.09 | 30.04 |
| 1293 | Lavandulyl acetate | 1.40 | 1.81 | 1.58 | 1.62 | 2.95 | 2.45 | 1.18 | 2.10 | 1.56 | 0.19 | 1.85 | 0.92 | 0.19 | 2.95 | 1.69 |
| 1386 | Geranyl acetate | 0.33 | 0.53 | 0.44 | 0.27 | 0.85 | 0.50 | 0.34 | 1.00 | 0.64 | 0.33 | 0.60 | 0.48 | 0.27 | 1.00 | 0.51 |
| 1429 | β -Caryophyllene | 2.17 | 3.19 | 2.64 | 1.56 | 2.12 | 1.80 | 1.16 | 1.71 | 1.40 | 1.03 | 2.69 | 1.70 | 1.03 | 3.19 | 1.79 |
| 1460 | β -Farnesene | 0.75 | 1.17 | 0.89 | 1.14 | 1.81 | 1.42 | 0.69 | 1.04 | 0.85 | 0.81 | 1.48 | 1.09 | 0.69 | 1.81 | 1.10 |
| 1492 | Germacren D | 0.67 | 0.94 | 0.72 | 0.65 | 0.83 | 0.74 | 0.45 | 0.67 | 0.54 | 0.38 | 0.83 | 0.59 | 0.38 | 0.94 | 0.64 |
| 1594 | Caryophyllene oxide | 0.09 | 0.19 | 0.13 | 0.06 | 0.13 | 0.08 | 0.05 | 0.12 | 0.08 | 0.00 | 0.16 | 0.11 | 0.00 | 0.19 | 0.09 |

^aRI: Retention indices on HP-5 capillary column.**Figure 1.** Raman spectra of lavender (FI) and lavandin (GR) EOs.

using literature data.^[13,28–30] The six major compounds showed a very strong band between 1446 and 1450 cm^{-1} (δ_{CH_2}). Borneol,

eucalyptol and camphor showed an intensive band at 652 cm^{-1} which could be assigned to the six-membered ring vibration with

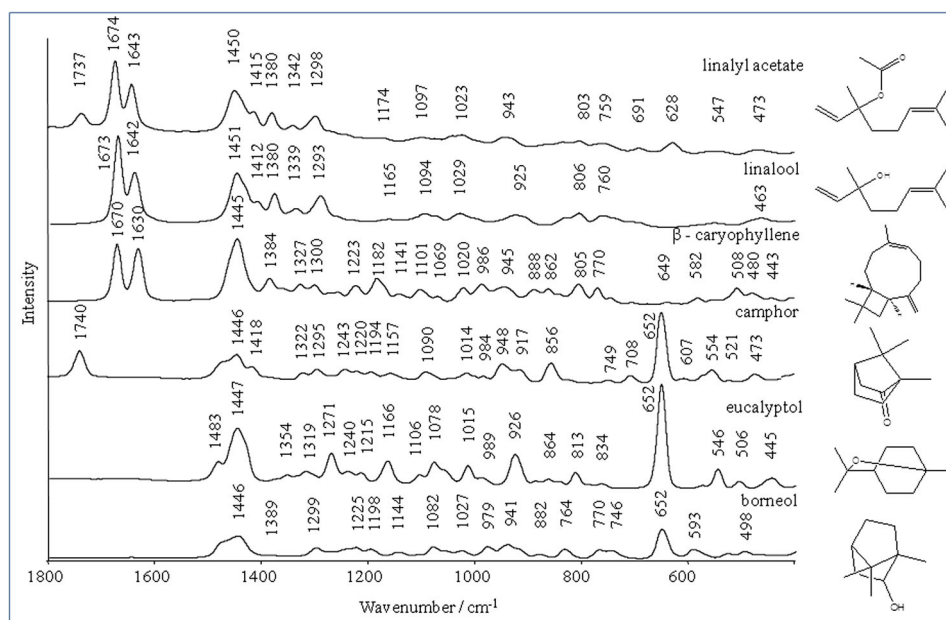


Figure 2. Pure spectra of the six main compounds in lavender and lavandin EOs.

an additional band for borneol at 835 cm^{-1} . β -Caryophyllene spectrum showed δ_{CH} and δ_{CH_2} at 885 and 862 cm^{-1} and weak bands for δ ring at 805 and 770 cm^{-1} . The asymmetric and symmetric $\nu_{\text{C}=\text{O}}$ appeared at 1214 and 1078 cm^{-1} respectively for eucalyptol, 1174 cm^{-1} for linalyl acetate and 1165 cm^{-1} for linalool. Linalool, linalyl acetate and β -caryophyllene spectrum were characterized by strong key bands including $\nu_{\text{C}=\text{CH}}$ and $\nu_{\text{C}=\text{CH}_2}$ at 1674 – 1677 and 1643 – 1645 cm^{-1} , respectively. Camphor and linalyl acetate showed a $\nu_{\text{C}=\text{O}}$ at 1740 and 1737 cm^{-1} respectively. The spectra of lavender and lavandin EOs presented profiles similar to that of their main compounds. The band at 1736 – 1738 cm^{-1} is representative of linalyl acetate and camphor. The two bands at

1673 and 1640 cm^{-1} are characteristic of linalool and linalyl acetate. The shoulder band at 1630 cm^{-1} characterizes β -caryophyllene.

Principal component analysis (PCA)

PCA was carried out on DRS data, and the result is shown in Fig. 3. The first two principal components represent 88% of the total spectral variance. The lavender and lavandin groups are clearly separated. They are differentiated on the first two components: lavender is negatively projected on the second while lavandin is projected positively on the first, and vice versa. The observation of the first component shows a negative contribution at 652 cm^{-1}

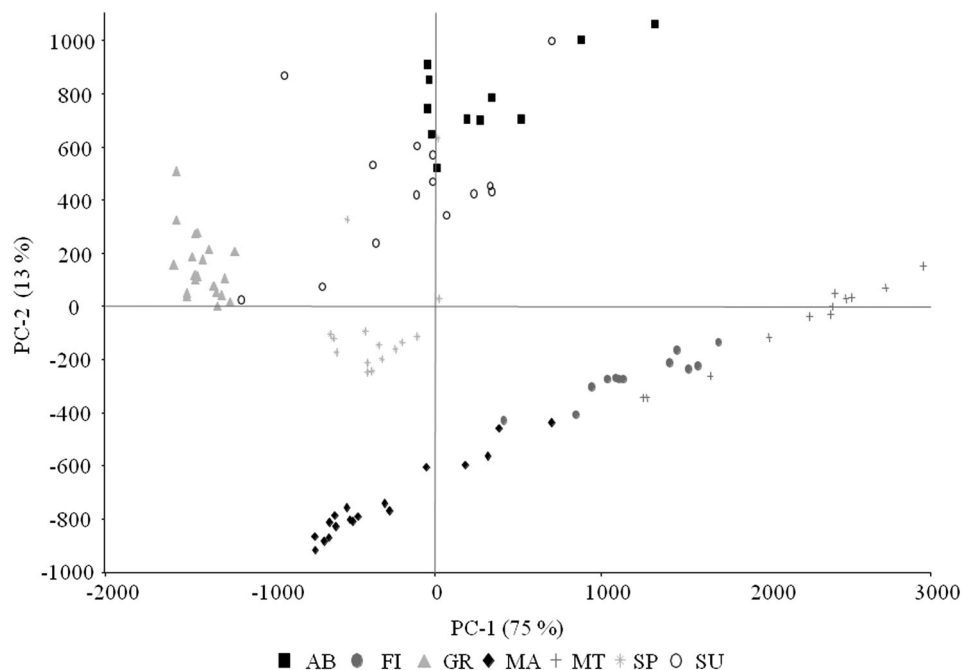


Figure 3. Score plot (PC1/PC2) of the PCA of Raman spectroscopy data ($n = 104$). Lavender EOs: Fine (FI), Maillette (MA) and Matherone (MT) and lavandin EOs: Abrial (AB), Grosso (GR), Super (SP) and Sumian (SU).

and another smaller contribution at 1740 cm^{-1} . The second component shows a high positive contribution at 652 cm^{-1} and other smaller contributions at 1740 cm^{-1} and 856 cm^{-1} , which were attributed to camphor according to the spectra of the pure compounds in Fig. 2. Camphor is a metabolic marker of lavandin species which have higher camphor content. The varieties of

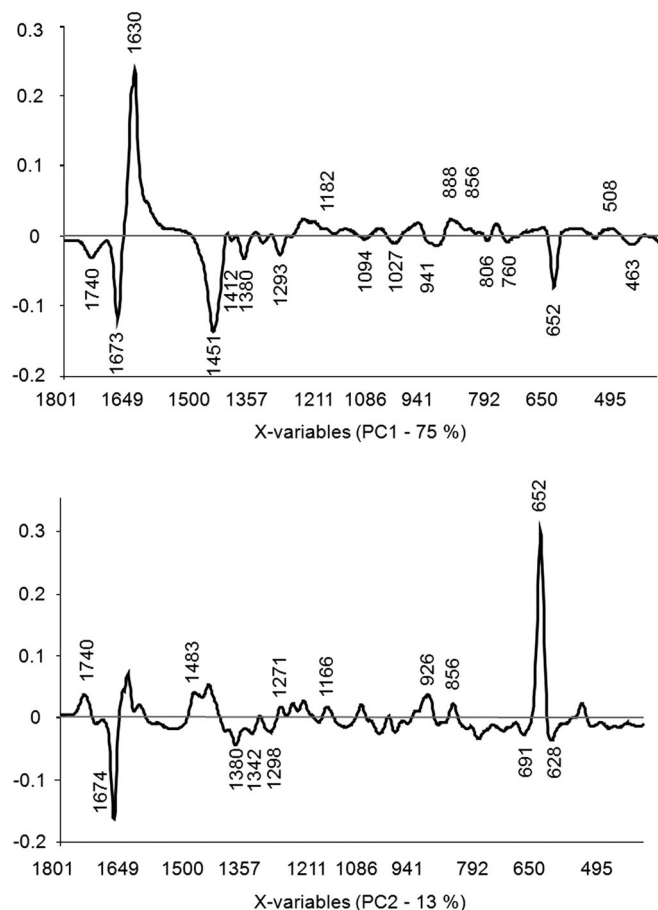


Figure 4. Loading plots (PC1/PC2) of the PCA of Raman data.

lavender and lavandin EOs were differentiated by the two components. The examination of the first component shows high negative contributions at 1673 cm^{-1} and 1451 cm^{-1} and other smaller contributions at 1412 , 1380 , 1293 , 1094 , 806 , 760 and 463 cm^{-1} attributed to linalool. These bands are anti-correlated with linalool concentration in the variety Matherone in which linalool is in very low concentration. The presence of other small negative contributions at 1027 and 941 cm^{-1} and one high contribution at 652 cm^{-1} was also noted. The association of these bands can be attributed to borneol. The band at 652 cm^{-1} can be attributed to camphor and borneol, the differentiation between these two compounds is on the bands at 1740 cm^{-1} for camphor and 1027 cm^{-1} for borneol. These bands are anti-correlated with the concentration of borneol and camphor in the varieties Matherone and Fine for which borneol and camphor are in very low concentration. The first component shows a high positive contribution at 1630 cm^{-1} , and other smaller contributions at 1182 , 888 and 508 cm^{-1} attributed to the β -caryophyllene. These bands are correlated with the high concentration of β -caryophyllene in the varieties Matherone and Fine and with the very low concentrations in the varieties Grosso, Super and Sumian. The second component shows smaller positive contributions at 1483 , 1271 , 1166 and 926 cm^{-1} attributed to eucalyptol (Fig. 4). The intense band at 652 cm^{-1} can be attributed to camphor and eucalyptol. These bands are correlated with the high concentration of eucalyptol in the variety Abrial and with the low concentration of eucalyptol in the variety Maillette. The second component shows a high negative contribution at 1674 cm^{-1} and other smaller contributions at 1380 , 1342 , 1298 , 691 and 628 cm^{-1} , attributed to linalyl acetate and correlated to higher concentrations in lavender oils.

Partial least square regression (PLS-DA)

The determination of essential oil compositions by chemometrics analysis of Raman spectra was achieved using PLS-DA regression algorithms. The reference data were gas chromatographic data. Table 3 gives the results obtained for the 14 quantified compounds (accounting for more than 0.4%). As shown in Table 3, compounds can be classified in three groups. In the first group, very good results were obtained for compounds having a high concentration, such as linalool and linalyl acetate with the REP <5%. In the second

Table 3. Statistics of the PLS regression models for the 14 main compounds in the lavender and lavandin EOs established from EMSC Raman data

| Compounds | Mean (%) | R^2 | SEC | LV | Q^2 | SEP | REP |
|---------------------------------|----------|-------|-------|---------------|-------|-------|-------|
| 3-Octanone | 0.59 | 0.98 | 0.069 | 15 | 0.90 | 0.217 | 36.78 |
| Limonene | 0.45 | 0.80 | 0.190 | 2 | 0.81 | 0.174 | 38.67 |
| Eucalyptol | 4.81 | 0.99 | 0.330 | 8 | 0.98 | 0.478 | 9.94 |
| <i>trans</i> - β -Ocimene | 2.36 | | | Not predicted | | | |
| Linalool | 33.70 | 0.99 | 0.794 | 7 | 0.98 | 1.467 | 4.35 |
| Camphor | 3.61 | 0.98 | 0.565 | 3 | 0.98 | 0.676 | 18.75 |
| Lavandulol | 0.64 | 0.90 | 0.255 | 10 | 0.85 | 0.452 | 70.62 |
| Borneol | 2.47 | 0.99 | 0.227 | 10 | 0.97 | 0.359 | 14.53 |
| Terpinen-4-ol | 1.72 | 0.99 | 0.132 | 12 | 0.99 | 0.198 | 11.51 |
| α -Terpineol | 0.86 | 0.95 | 0.094 | 12 | 0.89 | 0.171 | 19.88 |
| Linalyl acetate | 34.01 | 0.99 | 0.944 | 6 | 0.98 | 1.331 | 3.91 |
| Lavandulyl acetate | 2.34 | 0.98 | 0.321 | 7 | 0.93 | 0.648 | 27.69 |
| β -Caryophyllene | 3.23 | 0.96 | 0.463 | 6 | 0.93 | 0.616 | 19.07 |
| β -Farnesene | 1.61 | 0.97 | 0.160 | 13 | 0.80 | 0.396 | 24.60 |

R^2 : Coefficients correlation in calibration, Q^2 : Coefficients correlation in prediction, SEC: Standard error of calibration, SEP: Standard error of prediction, LV: Latent variables and REP: Relative error of prediction.

| | Species | Variety | Number of latent variables | AB (n = 3) | GR (n = 8) | SP (n = 5) | SU (n = 4) | FI (n = 4) | MA (n = 6) | MT (n = 4) | False-negative samples | False-positive samples |
|--------------|----------|---------|----------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|------------------------|------------------------|
| Raman models | Lavandin | AB | 9 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| | | GR | 11 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | SP | 13 | 1 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 2 |
| | Lavender | SU | 13 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 |
| | | FI | 8 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 1 | 2 |
| | | MA | 9 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| | | MT | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |

group, three compounds were correctly predicted because their relative error of prediction was $<15\%$ (eucalyptol, terpinen-4-ol and borneol). Compounds present in low content ($<5\%$) gave very poor results. The prediction of *trans*-ocimene content was impossible. Table 4 gives the prediction results for the 34 lavender and lavandin EOs recognition. The models were based on the same calibration and prediction set used for quantitative analysis. In step 1 the discrimination between the species was studied. The results were good, with 100% of correct classification. In step 2, the seven varieties were discriminated. Very good results were obtained with a percentage of correct classification between 91 and 100% as shown in Table 4. The varieties GR, MA and MT gave 100% of correct classification. Concerning lavender varieties, only the FI variety presented poorly classified samples with one false negative and two false positives. Regarding the lavandin varieties, AB presented one false positive, SU presented one false negative and SP presented two false positive samples. Raman spectroscopy was used as a recognition method. As it is possible to classify samples as a function of their species and varieties, it is interesting to understand how these classifications were established. As Haaland and Thomas

explained, the first coefficient of regression (noted B) was a good approximation of the pure compound spectrum^[31] in the case of PLS regression. Thus, the first coefficients of regression obtained for compounds were an approximation of the original feature of the species and varieties. For spectroscopists, those regression coefficients B were very useful to discriminate between metabolomic markers of lavender and lavandin essential oils. The first regression coefficients B obtained for the lavender and lavandin EOs (varieties) and those obtained for the quantitative analysis were compared. Figure 5 presents the superposition of the first regression coefficients B obtained for lavender varieties and their corresponding metabolomic markers, while Fig. 6 presents the superposition of the first regression coefficients B obtained for lavandin varieties and their corresponding metabolomic markers. For the lavender varieties, the FI first regression coefficient B is correlated with the regression coefficient obtained for quantitative analysis of lavandulyl acetate (Fig. 5a). The Pearson coefficient between the two first regression coefficients was 0.96. The FI variety presented a mean content of 4.34% in lavandulyl acetate. This high value shows that the FI variety is characterized by a high content in

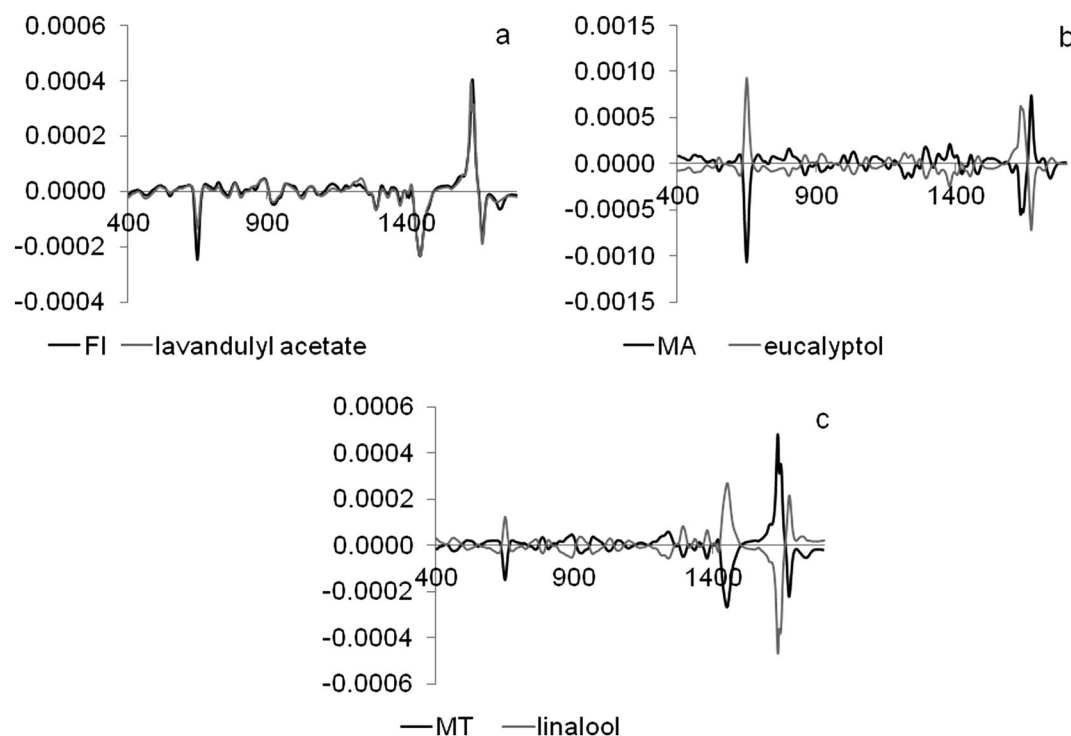


Figure 5. Superposition of the first regression vectors obtained for FI variety and lavandulyl acetate (a). Superposition of the first regression vectors obtained for MA variety and eucalyptol (b). Superposition of the first regression vectors obtained for MT variety and linalool (c).

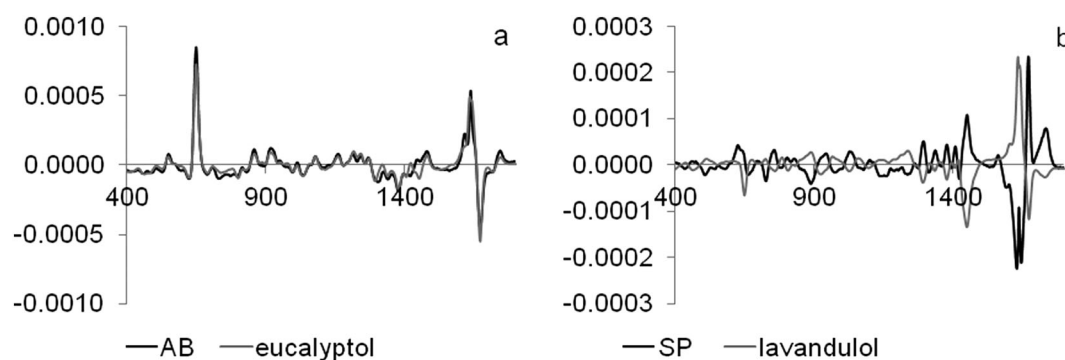


Figure 6. Superposition of the first regression vectors obtained for AB variety and eucalyptol (a). Superposition of the first regression vectors obtained for SP variety and lavandulol (b).

lavandulyl acetate. High concentrations of that compound were found in some samples of the MT variety. This could explain that two samples of the MT variety were classified in the FI group and the high mean content of the MT variety in lavandulyl acetate (5.17%). In conclusion lavandulyl acetate is a metabolomic marker for the FI variety. The MA first regression coefficient B presents high similarities and was anti-correlated with that obtained for eucalyptol quantitative analysis, with a Pearson coefficient of -0.97 (Fig. 5b). This high negative value showed that the MA variety was characterized by a low amount of eucalyptol. The MA variety presented a mean content of 1.36% in eucalyptol. This low content could be considered as a metabolomic marker of the variety. The MT first regression coefficient B is anti-correlated with that obtained for linalool quantitative analysis with a Pearson coefficient of -0.99 (Fig. 5c). The MT variety presented a mean content of 20.48% in linalool. This low content could be considered as a metabolomic marker of the variety. Concerning the lavandin varieties, the AB first regression coefficient B is correlated with that obtained on the rate of eucalyptol with a Pearson coefficient of 0.95 (Fig. 6a). The AB variety presented a mean content of 8.44% in eucalyptol. Eucalyptol is also a metabolomic marker for the AB variety but in that case at a high rate. The SP first coefficient of regression B is anti-correlated with that obtained on lavandulol quantitative analysis with a Pearson coefficient of -0.84 (Fig. 6b).

The SP variety presented a mean content of 0.32% in lavandulol. A low content in lavandulol could be considered as a metabolomic marker of the SP variety. No metabolomics markers were found for the GR and SU varieties.

Conclusion

Raman spectroscopy combined with chemometrics analysis can be used to quantify the main compounds in lavender and lavandin EOs. The prediction is reliable for compounds occurring in high concentration. For instance, camphor was correctly predicted, which is crucial to be able to detect adulterations. However low-content substances are difficult to predict with accuracy in essential oils. Raman spectroscopy combined with chemometrics analysis was used to discriminate between the species of essential oils studied (lavender and lavandin) and their varieties (Abrial, Grosso, Sumian, Super, Fine, Maillette and Matherone). The species and their varieties were predicted with accuracy (100% for species and between 91 to 100% for varieties). The examination of the first regression coefficient B allowed for identification of metabolomic markers for five varieties. Raman spectroscopy associated to

chemometrics analysis was demonstrated to be a powerful tool to confirm lavender authenticity. This approach could be applied to other essential oils commonly used in the perfume industry, in medicine and other fields.

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