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Chiroptical fingerprints to characterize lavender and lavandin essential oils

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A B S T R A C T

Polarimetric HPLC detector was used to acquire chromatograms for lavender/lavandin essential oils (EOs) on different chiral stationary phases. Amylose tris-(3,5-dichloro-phenylcarbamate) immobilized on silica allowed the best separation of the numerous chiral phytochemicals and was thus used to obtain a chiroptical fingerprint for 158 samples of French lavender/lavandin EOs. Samples from different varieties (Abrial, Fine, Grosso, Maillette, Matherone, Sumian and Super) were collected over three crop years (2012, 2013 and 2014), from four different locations in France ("Alpes-de-Haute-Provence", "Ardèche", "Drôme" and "Vaucluse") to take into account seasonal and geographic origin variations. The combination of the polarimetric fingerprints and chemometrics was tested for varietal discrimination. We assessed the ability to discriminate the EOs samples and their variety by partial least squares-discriminant analysis (PLS-DA). This case study showed that liquid chromatography with polarimetric detector in tandem with chemometric analysis was efficient to differentiate the varietal origins of French lavender/lavandin EOs.

Keywords:

Polarimetry
Chemometrics
Chiroptical detector
Lavender essential oil
PLS-DA

1. Introduction

The first polarimetric chromatogram obtained in liquid chromatography was reported in 1976 by Hesse and Hagel, who used a bench polarimeter to characterize the enantiomers of 2-phenylcyclohexanone separated on microcrystalline cellulose triacetate [1]. Since then, chiroptical detectors, i.e. polarimetric and circular dichroism (CD) detectors [2–4], were developed [5] and are nowadays routinely used in HPLC on chiral support to identify and differentiate chiral molecules [6]. Moreover, chiroptical detectors can be applied to the determination of enantiomeric ratios on chiral stationary phases (CSPs) with baseline [7] or partial separation [8,9], but also on achiral support [10–13], to preparative purposes [14,15], and to the assignment of absolute configurations [16–18]. They have sometimes been used to characterize complex mixtures focusing on chiral components [19], because the analysis is simplified by hiding achiral molecules, which are not sensed by chiroptical detection. For instance, sugars in human urine were quantified by polarimetric detection without chiral separation [20], enantiomeric ratios of lorazepam in human plasma were determined by

CD detection [21], and the absolute configuration of camphor was assigned in different *Artemisia* essential oils [22].

The chemical composition of an essential oil (EO) depends on the species, the origin, the cultivar, the harvest season, the extraction method and the nature of the vegetal material. The quality and the authenticity of EOs are assessed by the quantification of several compounds, according to the monographs produced by International Organization for Standardization (ISO) or European Pharmacopeia [23]. These chemical components are often chiral, so the use of chiral markers [24] and the determination of enantiomeric excesses are crucial for the characterization of EO [25,26]. The aim of this study was to use polarimetric chromatograms to characterize French lavender EOs. Indeed, lavender (*Lavandula angustifolia*) and lavandin (sterile hybrid of *Lavandula angustifolia* P. Mill. × *Lavandula latifolia* (L.f.) Medikus) are widely grown for their essential oils (EOs) in Mediterranean Basin [27]. The 39 *Lavandula* species and their numerous hybrids are of great interest in fragrance and flavor industries [28]: the lavender EOs containing larger amount of linalool and linalyl acetate are raw materials in perfumes and cosmetics, those with higher percentage of camphor are used for their anti-inflammatory and antibacterial properties, whereas lavandin EOs are widespread in household cleaners. These useful properties of lavender EOs are related to the properties of each enantiomer of each chiral component, so that they may be biased by the stereochemical composition of the EO. However, the

chiral compounds are not necessarily enantiopure in natural products [29], but they may occur as scalemic or racemic mixtures, thus indicating their formation by several biochemical pathways.

Lavender EOs are sometimes characterized by non-enantioselective techniques, such as gas chromatography [30], vibrational spectroscopies [31,32] or both [33]. Their chiral components can be studied, on one hand, by enantioselective GC, that is available since the early 90s [34], thanks to the enantioselectivity of cyclodextrin derivatives. Indeed, the separation by GC for the enantiomers of the main markers of lavender EOs has already been published [35–37] and recently optimized by flow modulated comprehensive two-dimensional GC to be used for quality control [38]. On the other hand, chiroptical vibrational spectroscopy has been used by our group to analyze the enantiomeric composition of the markers in *Artemisia herba-alba* EOs [39]. In the case of lavender EOs, preliminary tests proved that the intensity of the signal for vibrational and electronic circular dichroism spectra was too weak to be used.

To introduce an original way to characterize EOs, we focused on HPLC on chiral support with polarimetric detection. The principle of a polarimetric detector is to measure the angle of rotation of a linearly polarized light caused by the enantio-enriched molecule(s) in the cell, using one or several wavelength depending on the manufacturer. So, for a racemic mixture, two enantiomers separated on a chiral stationary phase (CSP), give signals of same area but opposite signs, positive and negative. The intensity of the signal is related to the value of the rotatory power at the wavelength(s) used by the polarimetric detector, to the amount and to the enantiomeric excess (ee) of the chiral sample. Liquid chromatography with polarimetric detection has already allowed the determination of enantiomeric excesses for a single EOs component, firstly isolated from the other molecules: for menthyl acetate in peppermint oils [40] and carvone in caraway or spearmint oils [41]. The injection of a crude EO on a HPLC column able to separate even partially several chiral molecules, leads to a polarimetric chromatogram with multiple positive and negative peaks, which can be considered as a chiroptical fingerprint of the mixture. Chemometrics analysis by Partial Least Squares-Discriminant Analysis regression (PLS-DA) of the polarimetric chromatograms was used to discriminate between the species of essential oils studied (lavender and lavandin) and their varieties (Fine, Maillette, Matherone, Abrial, Grosso, Sumian, Super). Lavender/lavandin EOs are good candidates because they contain mainly chiral compounds (80–90% of the total composition); (–)-(R)-linalyl acetate, (–)-(R)-linalool, (+)-(R)-camphor, (+)-(R)-borneol, (+)-(S)-terpinen-4-ol and (–)-(R)-lavandulol have been detected with high enantiomeric purities in lavender/lavandin EOs [36,42].

2. Material and methods

2.1. Essential oil samples

EOs obtained by steam distillation were provided by local French producers: 158 samples were analyzed including 94 lavandin oil samples and 64 lavender oil samples over 3 years of harvests (2012, 2013 and 2014), various varieties and various French collect areas (“Alpes-de-Haute-Provence” (04), “Ardèche” (07), “Drôme” (26) and “Vaucluse” (84)). The lavender/lavandin EOs studied were [*Lavandula angustifolia* Miller] (lavender) and its hybrid [*Lavandula angustifolia* Miller × *Lavandula latifolia* Linnaeus fil. Medikus] (lavandin) type France; they were divided into varieties: Fine (FI, $n = 21$), Maillette (MA, $n = 27$) and Matherone (MT, $n = 16$) varieties for lavender samples and Abrial (AB, $n = 16$), Grosso (GR, $n = 37$), Sumian (SU, $n = 19$) and Super (SP, $n = 22$) varieties for lavandin samples.

2.2. Pure standard substances

Pure standard substances were purchased from Alfa Aesar for (–)-borneol, (+)- and (–)-camphor; from Sigma Aldrich for (+)-borneol, (–)-limonene, β -myrcene, *cis*- and *trans*- β -ocimene, (–)-terpinen-4-ol and 3-octanone; from Fluka for (–)-linalool, racemic linalool, (+)-terpinen-4-ol and (+)-limonene; from Adrian for racemic lavandulyl acetate; from France lavande for racemic linalyl acetate; from TCI Europe for (–)- β -caryophyllene; and from Merck for eucalyptol, racemic α -terpineol and (–)- α -terpineol.

(–)-Linalyl acetate was synthesized from (–)-linalool (95% GC purity, 90% ee) by esterification in basic medium following Vidari procedure [43]. Then (–)-linalyl acetate was purified by preparative HPLC (>99% GC purity, >99% ee) with a Chiralpak IE column (250 × 10 mm, amylose tris-(3,5-dichloro-phenyl)-carbamate), Chiral Technologies Europe).

Racemic lavandulol (95% GC purity) was obtained from racemic lavandulyl acetate (94% GC purity) by hydrolysis in basic medium. (+)- and (–)-Lavandulol were then isolated by preparative HPLC (>96% ee for each enantiomer) with Lux-Cellulose-2 column (250 × 10 mm, cellulose tris-(3-chloro-4-methyl-phenyl)-carbamate), Phenomenex).

2.3. Chiral gas chromatography analysis

An Agilent Technologies gas chromatograph (7890A) was used with a flame ionization detector (FID), and a Rt@- β Dex_{se} capillary column Restek (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. 1 μ L of diluted essential oil (30 μ L in 1.5 mL of ethanol) was injected. Oven temperature was programmed 1 min at 40 °C, then 40 to 120 °C (1 °C.min⁻¹), then 120 to 170 °C (5 °C.min⁻¹), and held at final temperature for 20 min. Injector and detector temperatures were set at 220 °C. Hydrogen was the carrier gas at a flow rate of 4 mL.min⁻¹. Linear retention indices were calculated with reference to *n*-alkanes (C8–C24).

GC–MS analyses were performed on a 7890A GC system coupled to a 5975C VL mass spectrometer detector (Agilent Technologies) equipped with a Rt@- β Dex_{se} capillary column Restek (30 m × 0.25 mm, 0.25 μ m film thickness). Data acquisition and processing were performed using the MSD Chemstation E.01.01.335 (Agilent) software. 1 μ L of diluted essential oil (30 μ L in 1.5 mL of ethanol) was injected. The experimental conditions developed in the laboratory were the same that GC analysis except for injector and detector temperatures (250 °C); carrier gas (helium); ionization voltage 70 eV; electron multiplier, 1 kV.

Compound identification was done by comparison of their mass spectra with those of Wiley275 and NIST05a libraries as well as by comparison of their retention indices with those of authentic samples. EOs compositions were given as relative area percentages.

2.4. HPLC over chiral stationary phases

Different chiral stationary phases for HPLC were screened on Merck-Lachrom chromatographic units, controlled by the EZChrom Elite software, with a pump model L-2130, an autosampler model L-2200, an oven model L-2350 and a Jasco OR-1590 online polarimeter detector. The analytical solvents (heptane/isopropanol, 95:5, v/v, 1 mL min⁻¹, 25 °C) with HPLC grade are commercially available from VWR. They are degassed prior to use and filtered through Millipore membranes type HV/0.45 μ m. Essential oils were analyzed with a concentration of 10 mg/mL and the standards were recorded with a concentration of 3 mg mL⁻¹, both with 20 μ L as injection volume.

The 16 tested columns are Chiralpak IA, IB, IC, ID, IE, IF, AD-H, AS-H, AZ-H and Chiralcel OD-3, OJ-H (250 × 4.6 mm, Chiral Technologies Europe, Illkirch, France), Lux-Cellulose-2, Lux-Cellulose-4, Lux-Amylose-2 (250 × 4.6 mm, Phenomenex, Le Pecq, France) and (S,S)-Ulmo, (S,S)-Whelk-O1 (250 × 4.6 mm, Regis Technologies, Morton Grove, USA).

2.5. Chemometrics analysis

2.5.1. Data treatment

The HPLC data were transformed with linear baseline correction to remove baseline variation.

2.5.2. Principal component analysis (PCA) and partial least square regression (PLS)

Principal component analysis (PCA) and Partial least squares (PLS) regression are two tools currently used in chemometrics and were described in a previous study [31,33].

2.5.3. Software

Chemometrics analysis was performed using Unscrambler X V.10.3 (CAMO/Software, Oslo, Norway).

3. Results and discussion

After a classical determination of the EOs chemical composition by gas chromatography, the choice of chromatographic conditions compatible with the use of polarimetric detector is detailed. The chiral markers thus highlighted on a polarimetric chromatogram were used to discriminate between the varieties of lavender / lavandin EOs thanks to chemometrics.

3.1. Chemical and enantiomeric composition of the lavender and lavandin EOs studied

The lavender and lavandin essential oils contain 15 main molecules (see Supp. Data for chemical structures), including 10 chiral ones. Gas chromatography analyses on 2,3-di-O-ethyl-6-O-tert-butyl-dimethylsilyl β -cyclodextrin gave the chemical composition and the enantiomeric ratios of the 158 EOs according to the AFNOR norms (See Supp. Data for typical chromatograms and AFNOR norms). Compound identifications were done using retention indices and co-elution with standards of the 15 compounds investigated. Variations in composition, mean values and ranges are reported for lavender EOs in Table 1 and for lavandin EOs in Table 2.

Table 1

Chemical composition of lavender EO varieties (GC%).

Peak : RI ^a	Compounds	Fine (n=21)			Maillette (n=27)			Matherone (n=16)			Lavender (n=64)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
1 : 997	Eucalyptol, (+)- and (-)-limonene*	0.28	1.44	0.71	0.00	0.53	0.11	0.83	1.93	1.26	0.00	1.93	0.69
2 : 1009	β -Myrcene	0.30	0.93	0.50	0.00	1.65	0.43	0.33	0.83	0.54	0.00	1.65	0.49
3 : 1051	3-Octanone	0.37	1.59	0.88	0.89	2.56	1.59	0.17	0.88	0.43	0.17	2.56	0.97
4 : 1080	cis- β -Ocimene	2.72	7.72	4.90	1.04	4.18	1.95	4.15	8.73	6.68	1.04	8.73	4.51
5 : 1083	trans- β -Ocimene	2.15	5.19	3.70	0.48	4.19	1.31	6.85	14.38	9.42	0.48	14.38	4.81
6 : 1185	(+)-Camphor*	0.00	1.10	0.23	0.17	0.85	0.43	0.00	0.36	0.15	0.00	1.10	0.27
7 : 1211	(-)-Linalool*	19.33	32.94	28.51	24.36	53.73	41.65	13.11	27.07	20.36	13.11	53.73	30.17
8 : 1228	(+)-Linalool*	0.69	2.36	1.42	0.88	3.44	1.81	0.59	2.09	1.30	0.59	3.44	1.51
9 : 1244	(-)-Borneol* and (+)-borneol*	0.82	1.61	1.14	0.86	2.06	1.62	0.32	1.20	0.69	0.32	2.06	1.15
10 : 1270	(-)-Linalyl acetate*	37.11	48.51	41.90	34.27	54.89	43.66	38.72	49.42	42.89	34.27	54.89	42.82
11 : 1278	(+)-Terpinen-4-ol*	2.50	8.36	4.40	0.00	1.74	0.38	1.29	2.65	2.00	0.00	8.36	2.26
12 : 1287	(-)-Terpinen-4-ol*	0.00	0.29	0.03	0.00	0.21	0.01	0.00	0.27	0.05	0.00	0.29	0.03
13 : 1301	(-)-Lavandulyl acetate*	2.75	11.39	4.95	0.00	2.14	0.69	2.89	8.29	5.83	0.00	11.39	3.82
14 : 1320	(-)-Lavandulol*	0.56	2.26	1.07	0.00	1.42	0.18	0.56	4.49	1.63	0.00	4.49	0.96
15 : 1349	(+)- α -Terpineol*	0.00	0.93	0.45	0.00	1.18	0.53	0.00	0.94	0.46	0.00	1.18	0.48
16 : 1414	(-)- β -Caryophyllene*	3.72	6.26	5.21	2.76	6.16	3.63	5.27	7.35	6.32	2.76	7.35	5.05

^a RI: Retention indices on Rt@- β Dex_{se} capillary column.

(-)-Linalool (13.11–53.73%) and (-)-linalyl acetate (17.34–54.89%) were the main constituents and their enantiomeric composition was in accordance with literature [36,38,44]: linalyl acetate was found with high ee (>99%), while the two enantiomers of linalool were confirmed in all the varieties studied (ee between 88 and 94%). Enantiomeric distribution of linalyl acetate and linalool, the main compounds of lavender/lavandin EOs were already used to identify adulteration [45]. (+)-Camphor, (-)-lavandulyl acetate, (-)-lavandulol and (+)- α -terpineol were also found in high enantiomeric purity (ee>99% ee); their enantiomers (-)-camphor (retention indice RI: 1178), (+)-lavandulyl acetate (RI: 1309), (+)-lavandulol (RI: 1291) and (-)- α -terpineol (RI: 1342), have not been detected in EOs. This enantiopurity is a characteristic of Mediterranean EOs as already reported by Mosandl and coll. [36], and then by Flores et al. [44], but is lost in the Austrian Lavender oil sold by Sigma-Aldrich, as shown by Sandra and coll. [38], with only 87%, 84% and 36% ee for camphor, lavandulol and α -terpineol, respectively. In the case of terpinen-4-ol, the presence of the 2 enantiomers was observed (92 to 98% ee) except in AB and SP varieties where (+)-terpinen-4-ol has high enantiomeric purity (>99% ee). In our analytical conditions, eucalyptol, (+)- and (-)-limonene were co-eluted and (+)- and (-)-borneol were partially separated.

3.2. Choice of the HPLC stationary phase

An efficient description of an EO with a chiroptical detection requires multiple, thin and intense signals from its chiral components. For not enantiopure component, the use of chiral stationary phases rather than achiral ones doubled the number of potential observed peaks. Indeed, even in the case of very poor enantiomers separation, two opposite polarimetric signals should be distinguished [46,47]. Normal phase conditions on CSPs were chosen, allowing retention for the 8 oxygenated chiral compounds, which account for more than 90% of all chiral components: only limonene and β -caryophyllene were not retained. So, 16 different chiral stationary phases were screened in normal phase in order to obtain polarimetric traces containing as much information as possible, i.e. the maximum of positive and negative peaks. One sample of each variety of lavender/lavandin EOs (AB, FI, GR, MA, MT, SU and SP) was injected on the 16 CSPs and isocratically eluted with a binary mixture heptane / isopropanol (95:5, v/v) (See Supp. Data for the chromatograms of Abrial lavandin EO). On polarimetric detector, the first signals observed, between 3 and 3.5 min in our case, corresponds to the injection peak with unretained compounds that were not considered in this study. On each of the

Table 2
Chemical composition of lavandin EO varieties (GC%).

Peak : R_i^a	Compounds	Abrial ($n=16$)		Grosso ($n=37$)		Super ($n=19$)		Sumian ($n=22$)		Lavandin ($n=94$)			
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
1 : 997	Eucalyptol, (+) and (-)-limonene*	6.35	10.67	8.18	8.00	5.38	2.34	3.59	2.82	10.34	2.34	10.67	5.72
2 : 1009	β -Myrcene	0.35	0.58	0.49	0.81	0.59	0.41	1.13	0.63	0.64	0.35	1.13	0.56
3 : 1051	3-Octanone	0.14	0.37	0.24	0.00	0.11	0.44	1.17	0.76	1.47	0.00	1.47	0.48
4 : 1080	cis- β -Ocimene	1.23	3.33	2.62	1.23	1.66	1.26	3.06	1.83	4.55	1.23	4.55	2.30
5 : 1083	trans- β -Ocimene	0.49	5.09	3.20	0.15	0.46	1.01	2.79	1.75	3.08	0.15	5.09	1.81
6 : 1185	(+)-Camphor*	9.17	11.18	10.24	6.59	9.65	4.43	6.21	5.15	10.26	3.38	11.18	7.43
7 : 1211	(-)-Linalool*	33.81	40.20	36.76	30.10	40.81	31.12	40.50	36.42	50.94	30.10	50.94	38.51
8 : 1228	(+)-Linalool*	0.84	1.41	1.21	0.91	1.60	1.83	1.08	1.48	1.79	0.84	3.03	1.53
9 : 1244	(-)-Borneol and (+)-borneol*	2.48	3.71	3.21	2.67	4.55	2.46	4.56	3.59	8.68	2.39	8.68	4.19
10 : 1270	(-)-Linalyl acetate*	23.85	32.03	27.43	27.21	33.80	36.34	47.30	40.39	32.11	17.34	47.30	31.18
11 : 1278	(+)-Terpinen-4-ol*	0.54	1.60	0.99	2.09	3.78	0.00	1.02	0.31	3.86	0.00	5.52	1.46
12 : 1287	(-)-Terpinen-4-ol*	0.00	0.00	0.00	0.00	0.33	0.11	0.00	0.00	0.22	0.03	0.33	0.03
13 : 1301	(-)-Lavandulyl acetate*	1.41	2.09	1.72	1.62	2.60	0.83	2.27	1.55	0.00	0.00	3.27	1.62
14 : 1320	(-)-Lavandulol*	0.44	1.12	0.79	0.43	1.57	0.77	1.00	0.44	0.00	0.00	1.57	0.56
15 : 1349	(+)- α -Terpineol*	0.34	0.71	0.56	0.25	1.21	0.69	1.28	0.67	0.42	0.18	1.28	0.64
16 : 1414	(-)- β -Caryophyllene*	1.86	2.86	2.36	1.57	2.46	1.52	2.37	1.85	2.69	1.29	2.86	1.96

^a R_i : Retention indices on Rt@- β Dex_{se} capillary column.

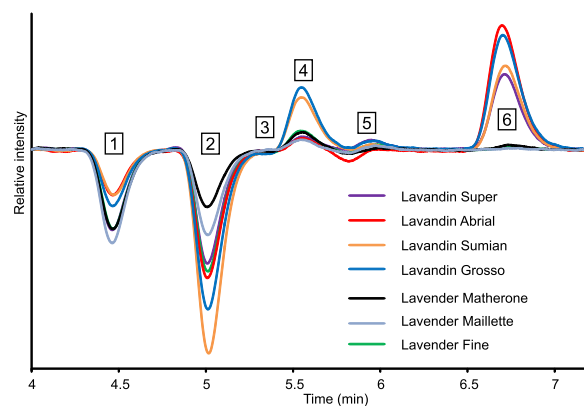


Fig. 1. Typical chromatograms of lavender/lavandin essential oils showing six characteristic areas using chiral HPLC (Chiralpak IE, heptane/2-PrOH 95/5, 1 mL min⁻¹, 25 °C) equipped with polarimetric detection.

16 chromatograms, two intense peaks, positive and negative, were distinguishable, more or less separated, with different elution order, accompanied by weaker signals on some columns. For instance, five distinct peaks were obtained between 4 and 7 min on Chiralpak IE, as shown in Fig. 1. Moreover, the intensities of these chiral markers vary depending on the lavender and lavandin EOs, so Chiralpak IE was chosen to pursue our goal to use polarimetric chromatograms to study French lavender EOs.

3.3. Identification of the chiral markers involved

For each pure standard substance, the polarimetric chromatogram was registered by injecting 60 μ g on Chiralpak IE with heptane/isopropanol (95:5, v/v) as mobile phase. Eucalyptol, 3-octanone, β -myrcene, *cis*- and *trans*- β -ocimene, the five achiral standards, gave no signal as expected. However, it was important to check that these compounds did not induce refractive index artefacts [48,49], i.e. pseudo-rotation signals caused by variation in the refractive index of the eluent, that might interfere with the study. Limonene and β -caryophyllene, the two non-oxygenated chiral molecules, were not retained in the normal phase conditions used. The first four minutes of the polarimetric chromatogram, corresponding to column dead time and to injection peak and to unretained compounds, were not considered in the chemometrics treatment.

The main chiral components were eluted between 4 and 7.2 min, their corresponding polarimetric chromatograms are overlaid in Fig. 2. In fact, the enantiomers of linalyl acetate, lavandulyl

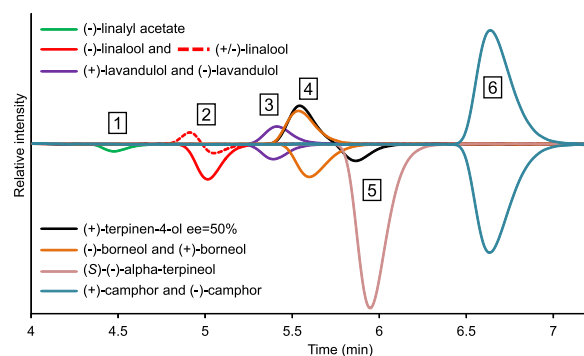


Fig. 2. Polarimetric chromatograms (Chiralpak IE, heptane/2-PrOH 95/5, 1 mL min⁻¹, 25 °C) of the main chiral standards components of lavender/lavandin essential oils: (-)-linalyl acetate, racemic linalool, (+)-lavandulol, (-)-lavandulol, (+)-terpinen-4-ol with ee = 50%, (-)-borneol, (+)-borneol, (S)-(-)- α -terpineol, (+)-camphor and (-)-camphor.

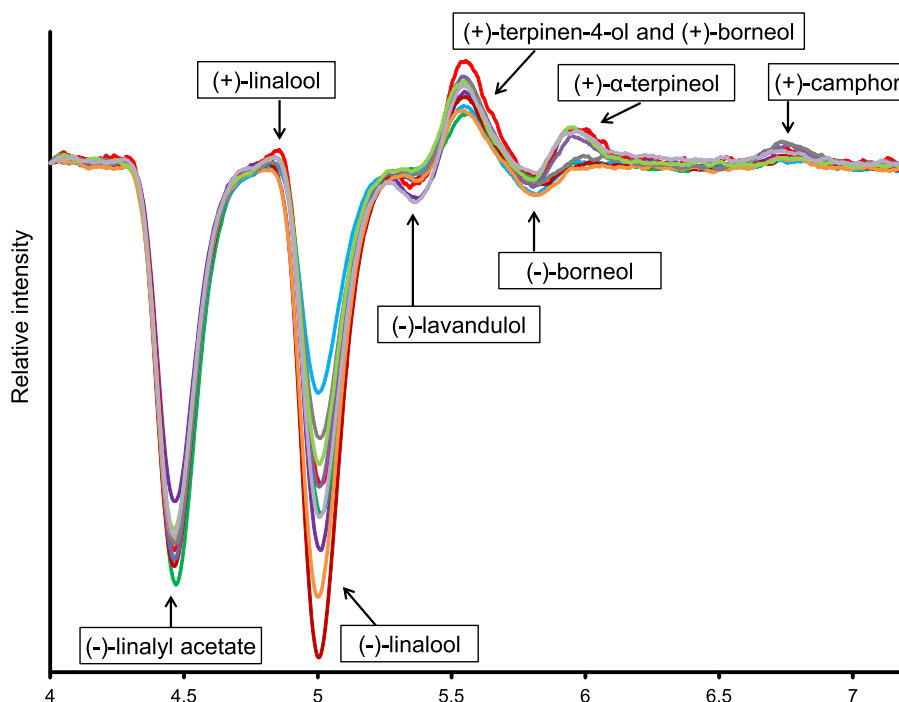


Fig. 3. Polarimetric fingerprints on Chiralpak IE for 10 different batches of Matherone lavender essential oils (heptane/2-PrOH 95/5, 1 mL min⁻¹, 25 °C).

acetate, lavandulol, α -terpineol and camphor were not separated on this chiral stationary phase. Chiralpak IE was only able to baseline separate (+) and (-)-terpinen-4-ol ($\alpha=1.19$), and poorly the enantiomers of linalool ($\alpha=1.13$) and borneol ($\alpha=1.03$). So, this method based on polarimetric detection did not require the enantiomers separation to discriminate between EOs in our case. Retention times, relative intensities and specific rotations at 589 nm from literature are given for the chiral standards in Supplementary Data. The intensity of the polarimetric signal given by the detector for an enantiomer depends on the magnitude of the optical rotatory dispersion. Indeed, the polarimetric detector used, Jasco OR-1590, has a multiple wavelength based operating mode: the signal results from the addition of the optical rotation contributions at wavelengths between 350 and 900 nm, the main contributions coming from the emission lines of the mercury lamp (365, 405, 436, 546 and 578 nm). This operating mode explains why the enantiomers with low specific rotation at 589 nm, like linalyl acetate or lavandulol, are easily detected and give workable signal. However, lavandulyl acetate has an optical rotatory dispersion that is too weak to obtain an available polarimetric trace.

Thanks to the seven remaining chiral components used as markers, six characteristic areas could be distinguished. For each area, the intensity of the observed sign was related to the amount, enantiomeric excess and optical rotatory dispersion of the corresponding compounds. The first area, labeled 1, shows a large negative peak for the EOs and corresponds to the co-elution of (+/-)-linalyl acetate, which have a small response but is present in large quantities, between 17 and 55%. Linalool is eluted in the area labeled 2, with a medium negative peak for enantiopure (-)-linalool but two separated positive and negative signals for the racemate. The intensity is lower for the racemate because the enantiomers are not baseline separated: the positive and negative peaks overlap, leading to a loss of polarimetric signal (Fig. 1). In the EOs, linalool is abundant, between 17 and 57%, and has a high enan-

tiomeric purity, (-/+)-ratio between 37/3 and 94/6: a large negative peak is so obtained in the area labeled 2 (Fig. 2). Lavandulol enantiomers are co-eluted in the area labeled 3, with a small polarimetric signal, due to its low specific rotation. Moreover, most of the EOs studied contains less of 2% of lavandulol, so no signal was observed in the area 3. It should be noted that for the only two Matherone lavender EOs with (-)-lavandulol contents of 4%, the negative polarimetric signal is clearly noticeable (Fig. 3), underlining the sensitivity of the method. In the area labeled 4, (+)-terpinen-4-ol, (+) and (-)-borneol are eluted. (+)-Terpinen-4-ol has a very high enantiomeric purity (ee > 99%) and its content in the EOs ranges between 0 and 8.36%. So that a positive peak is apparent on this area of the polarimetric chromatogram of each EO studied. Furthermore, this positive peak overlaps with the signal of (+)-borneol. Just following in this area, a small negative peak corresponds to the elution of (-)-borneol, which is thus evidenced in some Matherone lavender EOs (Fig. 3) and Abrial lavender EOs (See Supp. Data). The enantiomers of α -terpineol are co-eluted at 5.90 min in the area labeled 5. This component has a large polarimetric response but the (+)-enantiomer is present at less than 1.28% in the EOs studied, so that only a small positive peak is in this area. The area labeled 6 is characteristic of the amount of (+)-camphor in the EOs, since (+)-camphor is enantiopure and give a strong polarimetric signal between 6.5 and 7 min.

The polarimetric chromatogram obtained on Chiralpak IE can thus be considered as a chiroptical fingerprint of the EOs. Fig. 3 displays ten polarimetric fingerprints for different Matherone lavender EOs and allows comparing qualitatively their chemical and enantiomeric composition. For instance, the ten Matherone EOs differ according to the amount of (-)-linalyl acetate and (-)-linalool; some of them contains a small amount of (-)-linalool, (-)-lavandulol or (-)-borneol. These polarimetric fingerprints, showing significant variations between lavender/lavandin EOs, could be utilized for varieties differentiation.

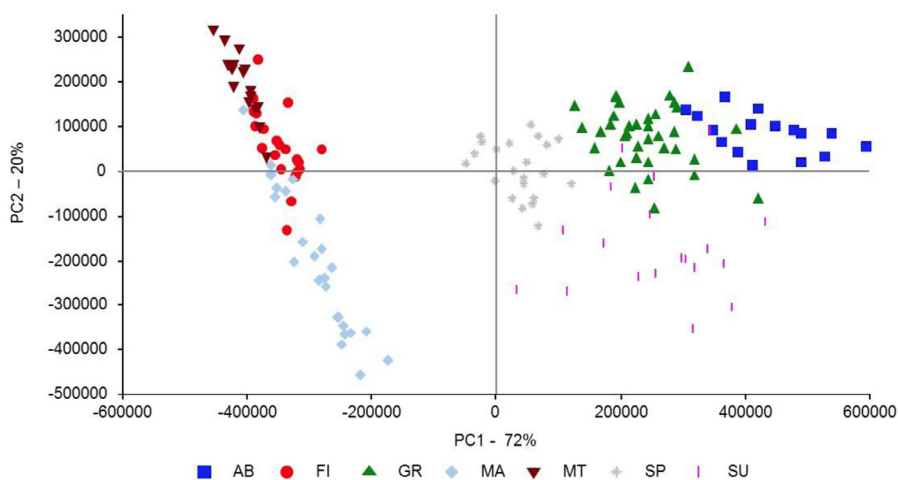


Fig. 4. Discrimination of lavender essential oils and lavandin essential oils. Score plot for the first and second components of the Principal component analysis (PCA) model of polarimetric fingerprints (Chiralpak IE column) with a linear baseline correction. AB: Abrial, FI: Fine, GR: Grosso, MA: Maillette, MT: Matherone, SP: Super and SU: Sumian.

3.4. Assessment of the differences in chemical composition by principal component analysis

The first and two components (PC1/PC2) of the PCA carried out on polarimetric fingerprints represent 92% of the total chromatographic variance (Fig. 4). The lavender and lavandin groups are separated on the first component (PC1), lavender is negatively projected while lavandin is positively projected except SP variety which is partially negatively projected but near the center. PC1 also allows the separation of lavender and lavandin varieties. PC1 can be described positively with linalyl acetate and camphor contents; and negatively with linalool content (Figs. 4 and 5). If we compare with GC data obtained previously [31], lavandin EOs have high (+)-camphor content, low (-)-linalyl acetate and high (-)-linalool content compared to lavender. MT variety has low (+)-camphor content (0.15%) compared to AB variety (10.24%). As it was observed previously, there is a gradient of camphor content in relation to PC1 (MT (0.15%) < FI (0.23%) < MA (0.43%) < SP (5.15%) < SU (6.65%) < GR (7.69%) < AB (10.24%). PC2 allows separation of SU variety and lavender varieties. PC2 can be described by linalool content. SU variety has high (-)-linalool content (45.25%). MT variety has low (-)-linalool content (20.36%) compared to MA variety (41.65%). As it was observed previously, there is a gradient of linalool content in lavender varieties in relation to PC2 (MT (20.36%) < FI (28.51%) < MA (41.65%).

3.5. Discrimination of lavender/lavandin EOs by PLS1-DA

The prediction results are given in Table 3 for the 52 lavender/lavandin EOs recognition. To build all PLS1-DA models, 106 samples were used: (FI, $n = 14$), (MA, $n = 18$), (MT, $n = 10$), (AB, $n = 11$), (GR, $n = 25$), (SU, $n = 15$) and (SP, $n = 13$) and the validation step was performed by full cross-validation. To test the performance of the models in prediction, 52 samples were used: (FI, $n = 7$), (MA, $n = 9$), (MT, $n = 6$), (AB, $n = 5$), (GR, $n = 12$), (SU, $n = 6$) and (SP, $n = 7$). For best prediction performance and to classify samples, the PLS1-DA regression models were built from linear baseline polarimetric fingerprints. In the step 1, the discrimination between the lavender/lavandin EOs was studied. The results were good, with 100% of correct classification with only 4 latent variables. In the step 2, the seven varieties were discriminated. Very good results were obtained for AB, GR (one false positive sample), SP (one false negative sample) and MA varieties with a percentage

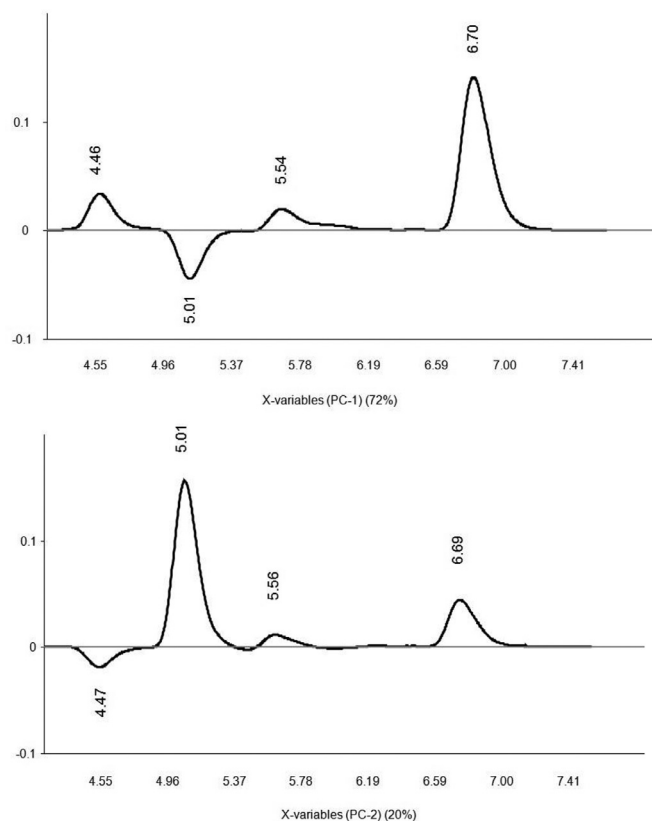


Fig. 5. Discrimination of lavender/lavandin essential oils. Loading plot for the first and second components of the Principal component analysis (PCA) model of polarimetric fingerprints with a linear baseline correction.

of correct classification between 96 and 100%. Good results were obtained for SU (two false negative samples) and MT (two false negative samples) varieties with a percentage of correct classification equal to 93%. Finally, FI variety (three false negative samples and one false positive sample) gives a percentage of correct classification equal to 86%. This low result can be explained by the fact that this variety comes from the lavender plant which grows spontaneously also called “wild *lavandula*”.

Table 3
Classification matrix obtained in prediction (PLS-DA) of lavender and lavandin varieties established from linear baseline polarimetric fingerprints (Chiralpak IE column).

Variety	Number of latent variables	Number of samples						False - positive samples		
		AB (n=5)	GR (n=12)	SP (n=7)	SU (n=6)	FI (n=7)	MA (n=9)	MT (n=6)	False - negative samples	False - positive samples
lavandin	AB	5	0	0	0	0	0	0	0	0
	GR	0	12	0	1	0	0	0	0	1
	SP	0	0	6	0	0	0	0	1	0
lavender	SU	0	0	0	4	0	0	0	2	0
	FI	0	0	0	0	4	0	1	3	1
	MA	0	0	0	0	0	9	0	0	0
	MT	0	0	0	0	0	0	4	2	0

4. Conclusion

Polarimetric detection combined with chemometric analysis enabled to differentiate lavender and lavandin essential oils and their varieties (Abrial, Grosso, Sumian, Super, Fine, Maillette and Mathéron), which were very well predicted with a percentage of correct classification of 100% for lavender/lavandin and between 93 and 100% for their varieties except for FI variety (86%), considering each lavender plant as a variety. According to chemometric analysis, the varieties are mainly discriminated by their content of (+)-camphor and (–)-linalool. The analysis time takes 8 min with HPLC, but could be shortened by using UPLC. This work also emphasizes the usefulness of chiroptical detectors whose sensitivity can be further improved to extend their scope, and the interest to develop UPLC chiroptical detectors.

Declaration of Competing Interest

None.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.chroma.2019.460568](https://doi.org/10.1016/j.chroma.2019.460568).

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