Parallel dual secondary-column-dual detection comprehensive two-dimensional gas chromatography: a flexible and reliable analytical tool for essential oils quantitative profiling

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Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) coupled with mass spectrometry (MS) is one of the most powerful analytical techniques now available for detailed analysis, identification, and quantitation of medium-to-high complexity mixtures. However the number of application methods that combine fingerprinting and/or profiling with quantitation of informative volatile analytes (targets) is still limited. Possible reasons are related to the huge amount of information to handle and to the availability of reference standards for calibration. Although quantitative analysis by GC×GC is complex it has important advantages: (*a*) to assess data/results over an extended time frame, varied instrumentation, and different laboratories; (*b*) to interpret the biological role of (potential) biomarkers; (*c*) to evaluate the impact of potent odorants; and/or (*d*) to define product quality, *e.g.*, relative to a reference standard.

In this study, a GC×2GC-MS/FID platform consisting of one primary column (^{1}D) coupled to two parallel secondary columns (^{2}D) having identical inner diameter, stationary phase chemistry, and film thickness, which, in turn, are connected to two detectors: a fast quadrupole MS and a FID, was adopted for quantitative profiling of essential oils (EOs).

Two medium complexity EOs (*i.e.*, *Mentha* and *Lavandula* species) that pose different quantitation challenges were taken as examples and a selection of quality markers subjected to an extensive method performance evaluation (*e.g.,* method validation). Experimental results confirmed the platform's reliability in terms of: linearity, precision, and quantitation accuracy. In addition, predicted FID Relative Response Factors (RRFs) based on combustion enthalpies were adopted to extend quantitation to all identified analytes. The experimental data demonstrated the accuracy of the predicted RRFs, supporting their adoption in quantitation of EO markers. This approach is of particular interest for those applications where reference standards are not (easily) available and/or regulated.

Key-words:

Comprehensive two-dimensional gas chromatography-mass spectrometry; parallel dual secondary column-dual detection; essential oil analysis; global quantitative profiling; Predicted Relative Response Factors

Introduction

Comprehensive two-dimensional gas chromatography (GC×GC) coupled with mass spectrometry (MS) is one of the most powerful analytical techniques now available for detailed analysis, identification, and quantitation of medium-to-high complexity mixtures. Compared to one-dimensional systems, GC×GC applies different selectivity in two chromatographic dimensions to provide higher separation power and unmatched peak capacity [1,2] combined with meaningful 2D elution patterns that facilitate analyte identification and sample fingerprinting. However, as reviewed by Marriott *et al.* [3] and more recently by Cordero *et al*. [4], the number of application methods that combine fingerprinting and profiling with quantitation of informative volatile analytes (targets) is still limited. A possible reason is that by increasing the number of analytes subjected to quantitation, the nature and amount of information to handle exponentially increases. The validation process for multi-analyte quantitative methods requires intensive data elaboration and extensive automation; otherwise, the benefits of single-run quantitation with GC×GC could be compromised by cumbersome and time-consuming data treatment. An example is linearity assessment, for which multi-analyte calibration operations must cover multiple concentration ranges (which sometimes are not contiguous), so accuracy should be validated over a wide range of concentrations where the matrix can differently exert detrimental effects on quantitation.

Although quantitative analysis by GC×GC is complex and time-consuming, it has important advantages, even when not mandatory [5,6]: (*a*) to assess data/results over an extended time frame, varied instrumentation, and different laboratories; (*b*) to interpret the biological role of (potential) biomarkers [7]; (*c*) to evaluate the impact of potent odorants on overall food aroma [8]; and/or (*d*) to define product quality, *e.g.*, relative to a reference standard.

For complex samples which may exhibit hundreds of potentially informative peaks, as is the case for some essential oils (EOs), the possibility of an extended/full quantitative assessment is attractive. However, quantitation by external standard calibration or by Relative Response Factors (*RRFs)* [9-13] may not be practical because of: (*a*) the lack of authentic/reference standards, and (*b*) the time required for multiple-compound calibration (as discussed above).

Most quantitative methods validated for targeted analysis by GC×GC are based on MS detection (high frequency Time-of-Flight MS (ToFMS) or fast quadrupole MS (qMS)) because this approach commonly is mandatory for regulated substances (xenobiotics, residues, contaminants, suspected allergens, *etc.*). In addition, for trace and ultra-trace analytes, as with several aroma compounds present in a food at sub-mg/Kg level [9,14,15], with MS detection, diagnostic ions can be used to increase Signal-to-Noise (S/N) ratios and thus method sensitivity.

In the EOs analysis, the challenges of quantifying the large number of peaks generated by GC×GC can be overcome, for non-regulated analytes in sample quality assessment, by adopting Predicted Flame Ionization Detector (FID) RRFs based on combustion enthalpies and molecular structure. This approach enables

quantitation without external standards. It was introduced by de Saint Laumer *et al.* [16] and applied to different quantitation problems by Tissot *et al.* [17], in particular for quantitation of markers of bergamot EO, purity assessment of unstable or reactive analytes, and profiling of dynamic mixtures stored at different temperature and pH conditions.

Predicted FID RRFs have been validated for GC×GC-FID applications by quantifying model mixtures of interest in the fragrance field by Tissot *et al.* [17] and, more recently, by Filippi *et al.* [18] for characterizing vetiver EOs from different geographical origins by GC×GC-MS and GC×GC-FID.

Because of the complementary attributes of FID responses and MS information, a GC×GC analytical platform that employs both types of detectors has exciting potential and so is of great interest. The concurrent presence of the two detectors operating on different principles, not only provides data for simultaneous analyte identification and quantitation, but also facilitates internal cross-validation of results [7]. The alignment of the separation patterns obtained with the two detectors at the data elaboration level allows unified consideration of the resulting data set from the integrated system.

A GC×2GC-MS/FID platform therefore has been implemented, inspired by previous papers from other researchers [18,19,20] with one primary column (^{1}D) coupled to two parallel secondary columns (^{2}D) having identical inner diameter, stationary phase chemistry, and film thickness, which, in turn, are connected to two detectors: a fast quadrupole MS and a FID [22]. Cryotrapping and refocusing is achieved with a dual-stage, loop-type thermal modulator at the head of the ^{2}D columns.

Unlike previous GC×GC platforms with a single ^{2}D column and dual parallel detection (MS/FID or MS/SCD), the adoption of two parallel secondary columns enables the system to operate at closer-to-optimal ^{2}D linear velocities and to double ^{2}D loading capacity, with positive effects on overall system orthogonality, resolution, and peak capacity. This last characteristic is fundamental when FID detection is used for quantitation purposes. With FID, quantitative accuracy requires highly resolved peaks; co-elutions generate quantitation errors that can be solved by adding a further system informative dimension, as that provided by MS. Previous studies on model solutions (homologue series of linear hydrocarbons and suspected volatile allergens) and on a medium-complexity EO (*Artemisia umbelliformis* Lam.) indicated that the GC×2GC-MS/FID provides consistent results, both in terms of analyte identifications (*e.g.*, reliability of spectra and MS matching) and the peaks' quantitative descriptors (*e.g.*, number of scans-per-peak and precision) [22].

In the present study, the GC×2GC-MS/FID platform is subjected to an extensive performance evaluation, with a focus on the method's accuracy and quantitation reliability, to evaluate its potential for the quantitative profiling of EOs. Two medium complexity EOs (*i.e.*, *Mentha* and *Lavandula* species) have been taken as examples that pose different quantitation challenges. *Mentha* spp. EOs were selected because of the presence of high abundance components (30-70 g/100g) closely eluting to informative quality markers that are present at low concentrations (0.1 g/100g), thus interfering with correct multi-target quantitation.

In addition, quantitative assessment of *Mentha* spp. EOs by 1D-GC-MS and GC-FID in a previous study [12] provides reference data for the current method validation and for more reliable evaluation of the system's potential. On the other hand, *Lavandula* spp. EOs were chosen because of the challenging quantitation aspects related to the complexity of the 2D patterns generated by adding a further informative dimension to the analytical platform, *i.e.*, the chiral recognition. The GC×2GC-MS/FID platform, in this case, is implemented by a ^{1}D Enantio-Selective (ES) stationary phase.

Target analytes included in the method validation process have been selected because of their role as markers for quality and/or botanical origin assessment. It is noteworthy that some of the selected analytes also recently have been mentioned in the Official Opinion of the EU Scientific Committee on Consumer Safety on "Fragrance allergens in cosmetic products" [23] and in a near future should be accurately quantified in cosmetics and/or fragrances to comply with regulatory requirements. Validation is focused on method linearity, precision, and accuracy against authentic reference standards. In addition, method suitability for full/extensive quantitation based on FID Predicted RRFs is verified.

Experimental

Essential Oils (EO) samples, reference standards for calibration and solvents

Pure standards of *n*-alkanes (from *n*-C9 to *n*-C25) for Linear Retention Indices (I^{T}_{S}) calibration, Internal Standard (ISTD) calibration, and Internal Quality Control (QC) verification were from Sigma-Aldrich (Milan, Italy).

Pure standards of 1,8-cineole, (*R*)-(*+*)-limonene, linalool, camphor, (*+*)-isopulegol, (-)-menthone, (*+*)-menthofuran, (*+*)-neomenthol, (2*S*)- (*+*)-borneol, lavandulol, menthol, (S)-(+)-4-terpineol, (*1S*,*2R*,*5R*)-(*+*)-isomenthol, (*R*)-(*+*)-pulegone, (*R*)-(-)-carvone, linalyl acetate, and menthyl acetate were from Sigma-Aldrich (Milan, Italy). (*R*)-(-)-lavandulyl acetate and (*+*)-neoisomenthol were from authors' laboratory.

Solvents (cyclohexane and dichloromethane) were all HPLC-grade, from Sigma-Aldrich (Milan, Italy).

Mentha x *piperita* L. EO (CS PEPP) was prepared in agreement to the method of the European Pharmacopoeia [24] and kindly supplied by Dr. Franco Chialva (ChialvaMenta, Pancalieri, Turin Italy). Commercial samples (CS) of *Mentha arvensis* L. (CS ARV), *Mentha spicata* L. (CS SPEAR), and *Mentha* x *gentilis* L. (CS GENT) EOs were purchased from the market.

Lavandula angustifolia Mill. (CS LAV01, CS LAV02, and CS LAV03) and *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik (lavandin Grosso) (CS GROSS) EOs were purchased from the market.

Calibration solutions and EO samples dilutions

Standard Stock Solutions of reference analytes were prepared at a concentration of 10 mg/mL in dichloromethane and stored at -18°C.

Calibration solutions for *Mentha* spp. EOs quantitation were prepared in dichloromethane by mixing suitable volumes of single component Standard Stock Solutions of 1,8-cineole, (*R*)-(*+*)-limonene, linalool, (*+*)-isopulegol, (-)-menthone, (*+*)-menthofuran, (*+*)-neomenthol, menthol, 4-terpineol, (*1S*,*2R*,*5R*)-(*+*)-isomenthol, (*+*)-neoisomenthol, (*R*)-(*+*)-pulegone, (*R*)-(-)-carvone, menthyl acetate, *n*-decane, *n*-undecane, *n*-dodecane, and *n*-tridecane. ISTD (*n*-Pentadecane) was included at a concentration of 25 mg/L.

Calibration solutions for *Lavandula* spp. EOs quantitation were prepared in cyclohexane by mixing suitable volumes of single component Standard Stock Solutions of (*R*)-(*+*)-Limonene, 1,8-cineole, linalool, 4-terpineol, camphor, (*R*)-borneol, lavandulol, linalyl acetate, (*R*)-(-)-lavandulyl acetate, *n*-decane, *n*-undecane, *n*-dodecane, and *n*-tridecane. ISTD *n*-Pentadecane was included at a concentration of 25 mg/L.

Calibration levels investigated were 5, 10, 15, 20, 50, 75, 100, 150, 200, and 250 mg/L for all reference compounds (including linear hydrocarbons *n*-C10 to *n*-C13 adopted for Internal QC).

GC-FID purity was controlled for each single component Standard Stock Solution before method validation and results are reported in **Tables 1** and **2** together with Target Ions (*Ti*) adopted for MS quantitation. Enantiomeric composition of chiral markers was determined by ES-GC×2GC-MS/FID and was considered for their calibration.

Mentha and *Lavandula* spp. EOs samples were prepared at different final concentrations to comply with the method linearity range. Final concentrations were as follows: 5, 2, and 1 mg/mL and 500 µg/mL and were obtained by diluting suitable volumes of a 10 mg/L EO Stock Solution in dichloromethane or cyclohexane. ISTD *n*-Pentadecane at a concentration of 25 mg/L was added to each analyzed sample.

GC×GC instrument set-up

GC×GC analyses were run with a system consisting of an Agilent 6890 GC unit provided with a 7683 ALS auto injector sampler (Agilent, Little Falls, DE, USA) coupled to an Agilent 5975C MS detector (Agilent, Little Falls, DE, USA) operating in EI mode at 70 eV. The GC transfer line was set at 280°C. A *Standard Tune* was used and the scan range was set to m/z 40-250 with a scanning rate of 12,500 amu/s to obtain a spectra generation frequency of 28 Hz. The Flame Ionization Detector (FID) conditions were: base temperature 280°C, H_2 flow 40 mL/min, air flow 240 mL/min, make-up (N_2) 450 mL/min, and sampling frequency 150 Hz. Injections of the EOs and of calibration mixtures, as well as those for I^T_S determination, were by 7683 ALS under the following conditions: split/splitless injector, split mode, split ratio 1/20, injection volume 1µL, and injector temperature 280°C.

Thermal modulator parameters

The system was equipped with a two-stage KT 2004 loop thermal modulator (Zoex Corporation, Houston, TX) cooled with liquid nitrogen controlled by Optimode™ V.2 (SRA Instruments, Cernusco sul Naviglio, MI, Italy). Hot jet pulse time was set at 250 ms; modulation time was 5 s; and cold-jet total flow was

progressively reduced with a linear function, from 40% of Mass Flow Controller at initial conditions, to 5% at the end of the run. Loop dimensions were chosen on the basis of the expected carrier linear velocities, to ensure that at least two stage-band-focusing releases were performed for each modulation. Thus, the first 0.6 m of the ^{2}Ds was wrapped in the metal slit of the modulator.

Column set, connections and auxiliary control module

The column set adopted for *Mentha* spp. quantitative profiling consisted of a primary column of 30 m × 0.25 mm d_c × 0.25 µm d_f SE52 (95% polydimethylsiloxane, 5% phenyl) connected to two secondary columns of equivalent length of 1.4 m × 0.1 mm d_c × 0.10 µm d_f OV1701 (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl). The oven temperature was programmed from 50°C (1 min) to 270°C at 3.0°C/min and to 290°C at 10°C/min (10 min).

For *Lavandula* spp. chiral recognition and quantitative profiling, the column set consisted of a primary column of 25 m × 0.25 mm d_c × 0.25 µm d_f of 6$^{I–VII}$-*O*-TBDMS-2$^{I–VII}$-3$^{I–VII}$-*O*-ethyl-β-CycloDextrin as chiral stationary phase (CSP) diluted at 30% in PS086 (DiEtβCD) [22] connected to two secondary columns of equivalent length of 1.4 m × 0.1 mm d_c × 0.10 µm d_f OV1701 (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl). The oven temperature was programmed from 60°C (1 min) to 180°C at 2.0°C/min and to 230°C at 10°C/min (5 min).

Connections between the primary and the two secondary columns were by a SilFlow™ GC 3 Port Splitter (SGE Ringwood, Victoria, Australia). The secondary column toward the MS detector was connected to a Quick Swap unit (G3185, Agilent, Little Falls, DE, USA) and to an auxiliary electronic pressure controller (EPC) consisting of a one channel Pneumatics Control Module (G2317A, Agilent, Little Falls, DE, USA). The restrictor capillary in the GC-MS transfer line was of 0.17 m x 0.1 mm d_c. A schematic picture of the system configuration is provided as a supplementary file (**Supplementary Figure 1 - SF1**). All columns and capillaries were from Mega (Legnano, Milan, Italy). The carrier gas was helium delivered at constant flow with initial head pressure p_i 296.0 KPa and the auxiliary gas for MS outlet pressure correction (He) was delivered at 39.9 KPa (relative). The split ratio (MS/FID) was 50:50. All technical aspects on columns' configuration, connections and auxiliary pressure corrections are discussed in detail in a previous paper by Nicolotti et al. [22].

Data acquisition and 2D data automatic processing

Data were acquired by Agilent MSD ChemStation ver D.02.00.275 and processed using GC Image® GC×GC Edition Software, Release 2.5 (GC Image, LLC Lincoln NE, USA).

Calibration curves were automatically generated within GC-Project® by applying a target template that included all calibrated analytes and ISTD. Each target compound in the template was characterized by its ^{1}D and ^{2}D retention times, *Ti* (Quantifier Ion for MS trace only), full mass spectrum (MS trace only), and

Qualifier CLIC® function to constrain template matching to a minimum MS spectrum similarity value match of 700 (NIST Similarity Match Factor and Reverse Match Factor).

Calibration curves, based on external standard responses normalized to ISTD, were generated from single calibration run images after target analyte identifications (template matching) and revision. Each calibration point was defined by 3 replicate runs, so that automatic processing on GC-Project® elaborated 24 runs (8 levels × 3 replicates) for each application (mint and lavender) and detector channel.

A linearity check was done for each detector signal (MS and FID) by arbitrarily fixing a minimum acceptable Coefficient of Determination (R^2) of 0.980. For analytes with $R^2 < 0.980$ calibration was adjusted by excluding external points (see **Tables 1** and **2**) but also including the zero level. Calibration curves for each concentration interval were saved as Calibration Table files and used, in GC-Image®, for automatic quantitation of EOs.

A detailed flow-chart of the automatic processing is available as **Supplementary Figure 2** (**SF2**).

Results and discussion

This section reports the validated quantitative results of GC×2GC-MS/FID applied to the analysis of two EOs taken as a model to evaluate this platform. It is divided into three steps: a) validation of the results taking 1D-GC-MS as a reference; b) peppermint GC×2GC-MS/FID analysis, in particular dealing with quantitation of minor components eluting close to major peaks; and c) combination of quantitative and enantioselective analysis of lavender EO markers.

Method Performance Parameters

Method validation was run over a four-weeks time interval to evaluate the following performance parameters: precision, linearity, and accuracy.

Precision data on retention times and on Normalized 2D Peak Volumes (*Ti* response for MS and total response for FID) were evaluated on three replicate analyses of calibration solutions during the entire validation period. Results on Normalized 2D Peak Volumes are reported in **Table 1** for *Mentha* spp. markers and in **Table 2** for *Lavandula* spp. markers, as average % Relative Standard Deviation (RSD%) values among replicates at each point.

Results demonstrate good precision on 2D Volume assessment for both detection channels with the FID showing better repeatability. Exceptions are menthol for the SE52/OV1701 stationary phase combination, which had an average RSD% on replicate analyses on all calibration points of 12.4 (MS) and 10.6 (FID), and (*R*)-(-)-Lavandulol on the DiEtβCD/OV1701 combination, which had an average RSD% of 11.7 for the MS channel. In general, FID gave more repeatable results compared to the MS detection and this trend is confirmed for both column configurations. A possible reason is related to the higher acquisition frequency of FID compared to fast quadrupole MS.

Linearity was assessed by linear regression analyses on Normalized 2D Peak Volumes *vs.* Relative Amount (ISTD *n*-Pentadecane was added at 25 mg/L) within the method working range (5-250 mg/L) and including the zero level. Each calibration curve includes at least six concentration points for each detector (*i.e.*, MS and FID) and column configuration. Zero level was included to facilitate quantitation of minor components (*e.g.*, minor enantiomers), although with a larger relative error.

Experimental results on linearity assessment for the SE52/OV1701 stationary phase combination are reported in **Table 1;** for DiEtβCD/OV1701, results are summarized in **Table 2**. Data reported include: calibration ranges; regression line slope, intercept and Coefficients of Determination (R^2); and Average Calibration Error % calculated on residuals at each calibration point.

Linearity was generally good with average R^2 of 0.993 and Average Calibration Error % always less than 20%. For EO markers quantitation, calibration curves were chosen on the basis of the analytes' expected concentrations and, when possible, confirmed over two different intervals. As for repeatability data, FID detection gave more stable responses resulting in lower quantitation errors. For this reason, the accuracy assessment was performed taking as "reference value" the FID quantitation result.

The accuracy was assessed by: (*a*) checking Internal QC analytes (*n*-alkanes from *n*-C10 to *n*-C13) spiked at a fixed concentration in all samples and (*b*) by cross-comparison of quantitative results obtained by MS and FID detection (linear regression gave R^2=0.998). In all cases, the Absolute Error, calculated as in Equation 1, was less than 20%:

$$(\text{Absolute Error \%})_i = |[(\text{MS Est. Conc.})_i - (\text{FID Est. Conc.})_i / (\text{FID Est. Conc.})_i] | * 100 \qquad \textbf{Eq. 1}$$

where $(\text{MS Est. Conc.})_i$ is the experimental concentration estimated within the method linearity range for analyte *i* from the MS signal and $(\text{FID Est. Conc.})_i$ is from the FID detection channel, arbitrarily considered as the reference value.

Accuracy results on *Mentha* and *Lavandula* spp. EOs are reported in **Table 3** and a more detailed and critical discussion on these data follows.

Quantitative profiling of *Mentha* spp. essential oils for accurate quality assessment

The QC of *Mentha* spp. EOs focuses on a series of authenticity markers requiring a quantitative profiling approach [26]. Area Percentage (Area %) of limonene, 1,8-cineole, menthone, menthofuran, isomenthone, menthyl acetate, isopulegol, menthol, pulegone, and carvone are assumed as quality markers in the European Pharmacopoeia [21], in the United States Pharmacopeia (USP), and in ISO Reference [27] for peppermint EOs (*Mentha* x *piperita* L., Lamiaceae). Isopulegol also plays an important role in the authentication and/or adulteration assessment of peppermint with *Mentha arvensis* L. (cornmint) [28,29]. Another quality marker of *Mentha spicata* (native spearmint) and *Mentha* x *gentilis* (scotch spearmint) species, (*R*)-(-)-carvone, is connoted by a distinctive odor note [30,31].

One-dimensional GC-FID and GC-MS methods perfectly fit with this purpose (*e.g.*, QC and authentication) and provide more reliable and robust results if based on "true" quantitation of reference markers (based on FID Response Factors - RF or External Standard Calibration - RRFs) instead of relative % abundance profiling [12,13]. However, when EOs are part of a more complex matrix, as in flavorings and/or fragrances, the resulting profile is more complex because of the presence of other components and the responses could be altered by matrix effect phenomena.

The validation results on the most informative markers for *Mentha* spp. EOs authentication (**Table 3**), confirm method quantitation reliability. The absolute quantitation error is less than 13% in all cases except for menthol in the *Mentha* x *gentilis* sample. These data are in agreement with and sometimes better than previous results [12]. A possible reason for more accurate quantitation by GC×GC is its higher separation power that for some analytes results in better chromatographic resolution. This aspect also was noted by Filippi *et al.* [21], who observed significantly different quantitative profiles for vetiver EO components when compared to the ISO reference pattern.

The 2D separation patterns of *peppermint* (CS PEPP) and *cornmint* (CS ARV) EOs in the elution region of menthols, shown in **Figures 1B** and **1C**, clearly illustrate how the higher ^{2}D column loadability due to the two-parallel ^{2}D columns, positively affects the overall system performance. For this group of analytes that show similar retention behavior on the ^{1}D stationary phase (*e.g.*, SE52), extra-chromatographic phenomena such as column overloading, would condition correct separation with detrimental effects on identification/quantitation of minor peaks eluting in the proximity of highly abundant components (*e.g.*, neomenthol and 4-terpineol *vs.* menthol above all). This critical cluster also is adequately resolved by 1D-GC with high efficiency columns (60 m long × 0.25 mm ID) coated with polar stationary phases (*e.g.*, Carbowax), as those recommended in the official methods [24].

Once quantitation consistency on reference analytes and external standard calibration is verified, the next step is the identification of EO constituents by matching MS spectra to those collected in commercial databases and verifying coherence of experimental I^{T}_{S} with tabulated ones. **Table 4** reports the list of identified analytes in *Mentha* spp. EO samples together with their retention times in the two chromatographic dimensions (^{1}D and ^{2}D Rt), experimental and tabulated I^{T}_{S}, and mass quantitative descriptors (*i.e.*, Normalized 2D Volumes %).

In the successive step, Normalized 2D Volumes (over ISTD *n*-pentadecane at 25 mg/L) from the FID detection channel of identified compounds were adopted for an extended quantitation based on Predicted RRFs.

Predicted RRFs were calculated according to the reference formulae [16,17] and normalized to *n*-pentadecane, here adopted as ISTD for normalization.

The Relative Response Factor equation is:

$RRF_i = 10^3 \ (MW_i/MW_{ISTD})(-61.5 + 88.8n_C + 18.7n_H - 41.3n_O + 3.8n_N, + 64.0n_S - 20.2n_F - 23.5n_{Cl} - 10.2n_{Br} - 1.07n_I + 127n_{benz}$ **Eq. 2**

where n_C, n_H, n_O, n_N, n_S, n_F, n_{Cl}, n_{Br}, n_I, and n_{benz} are the number of carbon, hydrogen, oxygen, nitrogen, sulfur, fluorine, chlorine, bromine, and iodine atoms, and the number of benzene rings, respectively. MW_i and MW_{ISTD} are the molecular weights of the analyte *i* and the ISTD adopted for the development of the model by de Saint Laumer *et al.* [16] (ISTD, methyl octanoate).

The analyte specific RRF was corrected to the *n*-C15/methyl octanoate ratio (*i.e.*, $RRF_{i,n\text{-}C15}=0.718/RRF_{i,methyl\ octanoate}$) to adapt the model to *n*-pentadecane.

Results of the extended quantitation of EO components are reported in **Table 4**. Relative quantitation differences indicate good accuracy of the predicted *RRF* approach for those analytes already quantified by external standard calibration.

Figure 2A shows the distribution of Relative Error % for *Mentha* spp. markers, taking the external standard quantitation approach as reference. Most compounds show good accuracy when quantified using predicted RRFs, although some, such as menthone (CS ARV and CS PEPP) and carvone (CS SPEAR), exceed 20% Relative Error. These data are in good agreement with Tissot *et al.* [17], who compared nominal concentrations of a reference mixture to those estimated by predicted RRF with GC×GC-FID through the Euclidean distances, and with Filippi *et al.* [18], who investigated a high-complexity EO (*i.e.*, Vetiver EO), taking as reference values those estimated by applying Response Factors (RF) specific for the different chemical classes.

In the perspective of quality assessment, experimental results confirm that the *Mentha* x *piperita* EO (CS PEPP) profile is in agreement with the European Pharmacopeia specifications for both: (a) markers' percentage areas distribution and (b) 1,8-cineole/limonene ratio (reference ratio ≥ 2) [24,28]. Isopulegol content (0.09 %) is in accordance with the authentic peppermint reference pattern [21]. The profiles of the investigated spearmint EOs show some quantitative differences between *Mentha* x *gentilis* and *Mentha spicata*. In any case, the chemical composition of *Mentha* x *gentilis* EO is comparable to that reported by Lawrence [33].

Quantitative Profiling and Enantiomeric recognition of *Lavender* spp. essential oils

Once system quantitation reliability with a medium-complexity EO *(e.g.*, *Mentha* spp.) was confirmed, a further dimension of information was included in the analytical platform, *i.e.*, the enantiomeric recognition of chiral markers, in particular, of lavender spp. EOs.

The QC of lavender spp. EOs focuses on a series of authenticity markers requiring a quantitative profiling approach [24]. Area Percentage (Area %) values and/or intervals are reported for linalool, linalyl acetate, lavandulyl acetate, 4-terpineol, lavandulol, 1,8-cineole, camphor, and borneol in the European

Pharmacopoeia [24] and in some ISO References [33,34] for *Lavandula angustifolia* Mill. and for *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. (lavandin Grosso).

Another important parameter for quality assessment of lavender EOs is the enantiomeric composition of the chiral components [35]. Among the diagnostic markers, linalyl acetate and linalool are both present in a high enantiomeric excess, greater than 99%, of the (*R*)-(-) form independent of variety, storage, and growth conditions [36].

Therefore, the quality assessment of lavender EO should include contemporarily chiral and achiral components quantitative profiling, requiring an analytical platform that combines enantiomeric recognition and high peak capacity suitable to handle the complexity of the resulting 2D pattern. This topic was investigated by Bicchi *et al.* [37,38] for 1D-(ES)-GC-MS, who concluded that a fine tuning of carrier gas linear velocity and temperature rate is mandatory to achieve the resolution of all chiral and achiral markers in a single injection.

When a GC×GC platform is adopted, carrier gas linear velocity in the two dimensions cannot be independently optimized and, in many cases, the higher peak capacity compensates for the sub-optimal carrier gas operative conditions. However, to exploit the ^{1}D chiral recognition by Cyclodextrines (CDs) properly, chromatographic parameters (carrier gas velocity and temperature) should be correctly matched to provide discrimination based on the small difference in the energy of the host/guest interactions between each enantiomer and the chiral selector. The GC×2GC-MS/FID platform operates close-to-optimal in both chromatographic dimensions enabling the full chiral recognition without loss in the overall peak-capacity. **Figure 3** shows the resulting 2D pattern of one *Lavandula angustifolia* Miller EO together with some critical sub-regions in which components coeluting in the ^{1}D are separated in the ^{2}D.

Quantitative results demonstrate fairly good accuracy (**Table 3**) with Absolute Error % values not exceeding 18%.

When quantitative profiling, based on predicted RRFs, is extended to all analytes identified on the basis of MS spectrum similarity and enantiomer specific I^{T}_{S} from a CDs dedicated database [39], accuracy is still acceptable; results are reported in **Table 5**. Relative Errors %, visualized in the histogram of **Figure 2B**, confirm the tendency to overestimate, compared to external standard calibration, already observed for *Mentha* spp. and, with some exceptions, indicate that predicted RRFs results are generally in agreement with those obtained by true quantitation. Interestingly, compounds that exceeded ± 20% error, such as 1,8-cineole and (S)-borneol, also exhibited lower accuracy values, although acceptable when considering the absolute concentration in the analyzed samples [40].

Experimental results on 2D Volume % confirm that the lavandin EO (CS GROSS) chemical pattern is in agreement with the ISO Reference [34], while within the *Lavandula angustifolia* samples only the CS LAV01 shows a profile compatible with the European Pharmacopeia reference [24]. On the contrary, CS LAV02 and CS LAV03 reports very high percentages of 1,8-cineole, borneol, and camphor, that are outside the range.

Enantiomeric distribution of linalool and linalyl acetate, detailed in **Table 6**, leads to exclude the addition of synthetic racemates to the EOs. In particular, the enantiomeric % composition (EC%) of (*R)*-(-)-linalyl acetate is always greater than 97.5%, whereas that of (*R*)-(-)linalool is generally smaller but still acceptable [24]. (*S*)-(*+*)-linalool up to 15% may be formed during unusual and extremely time-prolonged hydrodistillation processes [38].

Conclusions

The performance of a dual-secondary-column, dual-detection system in an integrated platform for GC×GC has been evaluated and critically discussed in view of its adoption for detailed EO quantitative profiling. In particular, extensive method validation confirmed the platform's reliability in terms of: linearity, precision, and accuracy. This last parameter was assessed by cross-matching quantitative results between the two detectors, *i.e.*, MS and FID. Although the MS detection adds to the system a further analytical dimension that enables unequivocal analytes identification and quantitation, FID response is related to its chemical structure and can be predicted with a reasonable accuracy by mathematical models [16]. When the molecular structure is known, the analyte amount in the sample can be estimated, making it possible to extend the quantitation to all identified compounds of a sample. The experimental data demonstrated the accuracy of the predicted RRFs, supporting their adoption in quantitation of EO markers. This approach is of particular interest in those applications for which reference standards are not (easily) available and/or regulated.

Challenging quantitation problems can be overcome thanks to the close-to-optimal ^{2}D linear velocities and doubled loading capacity of the 2nd dimension, as for instance: (a) the accurate quantitation of minor peaks eluting in the proximity of a major component, *e.g.*, neomenthol and 4-terpineol vs. menthol in peppermint and cornmint EOs, and (b) contemporary monitoring and quantitation of chiral and achiral markers without resorting to the MS dimension to resolve co-elutions, *e.g.*, linalool and linalyl acetate in lavender EOs.

The GC×2GC-MS/FID platform's quantitation reliability is therefore of high interest, because it matches several analytical needs, enabling an extended quantitative profiling of medium-to-high complexity matrices for both authentication and regulatory purposes. Therefore, its adoption is highly promising in view of its applications to matrices submitted to REACH regulation (Registration Evaluation Authorisation and Restriction of Chemicals) [41], to complex fragrances containing suspected allergens of the EU list, or for upcoming regulations [6,23,43].

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Caption to Figures

Figure 1: 2D plot of menthol-rich EOs. **Fig. 1A** peppermint (CS PEPP) 2D elution pattern, **Fig. 1B** elution region of menthols in peppermint (*Mentha x piperita* L. - CS PEPP) and **Fig. 1C** in cornmint (*Mentha arvensis* L. - CS ARV).

Figure 2: Relative Error % distribution for *Mentha* spp. (**Fig. 2A**) and lavender (**Fig. 2B**) EOs quality markers quantified through FID RRFs. External Standard quantitation by FID is taken as reference value for accuracy evaluation.

Figure 3: 2D plot of *Lavandula angustifolia* Miller EO (Fig. 3A - CS LAV01) obtained by DiEtβCD/OV1701 column combination. Two sub-regions are also shown (Fig. 3B - region *I* and Fig.3C - Region *II*) where components are effectively resolved in the ^{2}D.

Caption to Tables

Table 1: Validation results for *Mentha* spp. EOs quality marker quantitation by GC×2GC-MS/FID. Target analytes are reported together with GC-FID purity of reference compounds adopted for external quantitation, Target Ion (*Ti*) adopted for quantitation, calibration interval(s), regression parameters (slope, intercept and Coefficient of Determination R^2), precision (referred as average Relative Standard Deviation % - RSD% between replicates at each calibration point) on Normalized 2D Volume and residual distribution (reported as the Average Calibration Error % on all calibration points) for both detectors.

Table 2: Validation results on quality markers of lavender EOs by GC×2GC-MS/FID with DiEtβCD/OV1701 column combination. Target analytes are reported together with GC-FID purity of chiral reference compounds adopted for external quantitation, Enantiomeric Composition %, Target Ion (*Ti*) adopted for quantitation, calibration interval(s), regression parameters (slope, intercept and Coefficient of Determination R^2), precision (referred as Relative Standard Deviation % - RSD%) on Normalized 2D Volume and residual distribution (reported as the Average Calibration Error % on all calibration points) for both detectors.

Table 3: Quantitative results (expressed as g/100 g of EO) obtained by GC×2GC-MS/FID on *Mentha* spp. and lavender EOs under study. Absolute Error % is calculated taken as reference value that obtained by FID.

Table 4: List of identified analytes in *Mentha* spp. EO samples together with their molecular weight (MW) and formula, retention times in the two chromatographic dimensions (^{1}D and ^{2}D Rt), experimental and tabulated I^T_S, mass quantitative descriptors (*i.e.*, Normalized 2D Volumes %), estimated concentration (mg/100g) obtained by predicted FID RRFs and corresponding concentrations (when available) obtained by external standard quantitation.

Table 5: List of identified analytes in lavender EO samples together with their molecular weight (MW) and formula, retention times in the two chromatographic dimensions (^{1}D and ^{2}D Rt), experimental and tabulated I^T_S, mass quantitative descriptors (*i.e.*, Normalized 2D Volumes %), estimated concentration (mg/100g) obtained by predicted FID RRFs and corresponding concentrations (when available) obtained by external standard quantitation.

Table 6: Enantiomeric Composition % of lavender EO samples.

Table 1

			MS detection channel (Full-SCAN Mode)						FID detection channel				
Analyte	**GC-FID Purity % (SE52)**	***Ti* (MS Quantifier Ion)**	**Calibration Interval**	**Slope**	**Intercept**	**R^2**	**Precision Average Norm. 2D Vol (RSD%)**	**Average Calibration Error %**	**Slope**	**Intercept**	**R^2**	**Precision Average Norm. 2D Vol (RSD%)**	**Average Calibration Error %**
Limonene	98.5	93	10-250	0.97	-0.46	0.993	4.4	-10.2	1.17	-0.20	0.992	1.7	-9.3
			0-150	0.96	-0.32	0.994			1.16	-0.14	0.993		
1,8-Cineole	99.8	81	10-250	0.55	-0.11	0.990	6.2	-10.7	1.14	-0.51	0.997	3.6	-1.6
			0-150	0.55	-0.08	0.992			1.14	-0.74	0.989		
Linalool	99.8	71	10-250	0.68	-0.92	0.996	9.4	-5.5	1.18	-1.59	0.995	3.8	-4.5
			0-150	0.66	-0.64	0.993			1.15	-1.10	0.993		
4-Terpineol	96.3	81	10-250	1.15	-0.97	0.998	8.3	-12.0	0.90	-0.20	0.992	4.1	-11.0
			0-150	1.12	-0.68	0.997			0.90	-0.14	0.994		
Menthofurane	94.4	108	10-250	0.16	0.07	0.995	9.6	-6.7	0.88	-0.79	0.996	4.2	-10.7
			0-150	0.17	0.05	0.995			0.87	-0.55	0.995		
Menthol	99	71	10-250	0.94	-0.68	0.996	12.4	-10.5	1.14	-0.57	0.999	10.6	-4.8
			0-150	0.93	-0.47	0.996			1.13	-0.40	0.999		
Menthone	78.1	112	10-250	0.87	-0.77	0.999	6.5	-14.4	1.01	-0.36	0.993	7.6	-16.8
			0-150	0.85	-0.54	0.996			1.00	-0.25	0.994		
Menthyl acetate	92.7	138	10-250	0.38	-0.38	0.998	5.8	-14.4	1.06	0.08	0.992	6.2	-14.5
			0-150	0.37	-0.27	0.995			1.06	0.05	0.994		
Neoisomenthol	54.9	71	10-250	2.33	-0.82	0.994	8.8	-11.4	1.03	0.30	0.969	6.4	-14.2
			0-150	2.29	-0.57	0.994			1.03	0.21	0.968		
Neomenthol	99.8	71	10-250	0.19	-0.23	0.997	9.6	-8.2	1.14	-0.28	0.991	2.7	-6.8
			0-150	0.18	-0.16	0.995			1.14	-0.28	0.991		
Pulegone	98.3	81	10-250	1.04	-1.31	0.999	7.5	-9.3	0.83	-0.19	0.990	7.7	-11.9
			0-150	1.02	-0.91	0.996			0.82	-0.13	0.992		
Carvone	99.8	82	10-250	1.32	-1.62	0.998	8.9	-8.9	0.86	-0.43	0.995	3.6	-9.1
			0-150	1.28	-1.12	0.995			0.85	-0.30	0.996		
Isopulegol	99.5	67	10-250	0.31	-0.07	0.964	7.5	-19.3	0.89	-0.32	0.992	3.0	-9.4
			0-150	0.31	-0.05	0.966			0.88	-0.22	0.993		
Isomenthol	99.8	71	10-250	0.74	-0.71	0.995	8.3	-8.0	0.77	-0.40	0.993	4.9	-5.3
			0-150	0.72	-0.50	0.994			0.76	-0.28	0.994		
Decane	99.8	57	10-250	1.50	0.04	0.986	6.8	-12.5	1.10	-0.25	0.990	3.2	-9.4
			0-150	1.50	0.03	0.988			1.11	-0.17	0.992		
Undecane	99.8	57	10-250	1.45	0.01	0.987	6.1	-11.9	1.00	0.15	0.976	2.1	-12.1
			0-150	1.45	0.01	0.987			1.00	0.10	0.980		
Dodecane	99.7	57	10-250	1.67	0.10	0.985	5.1	-12.4	1.05	-0.14	0.989	2.2	-9.3
			0-150	1.67	0.07	0.988			1.04	-0.10	0.991		
Tridecane	99.3	57	10-250	1.56	-0.02	0.988	6.6	-12.3	1.00	-0.06	0.988	3.3	-9.9
			0-150	1.56	-0.01	0.990			1.00	-0.04	0.991		

Table 2

				MS detection channel (Full-SCAN Mode)						FID detection channel				
Analyte	**GC-FID Purity % (SE52)**	**ES-GC-FID Enantiomeric Composition % (βCD)**	***Ti* (MS Quantifier Ion)**	**Calibration Interval**	**Slope**	**Intercept**	**R^2**	**Precision Average Norm. 2D Vol (RSD%)**	**Average Calibration Error %**	**Slope**	**Intercept**	**R^2**	**Precision Average Norm. 2D Vol (RSD%)**	**Average Calibration Error %**
(*R*)-(+)-Limonene	98.5	99 (R)-(+)	93	10-250	0.98	-0.50	0.999	4.6	-9.1	1.17	-0.17	0.996	1.5	-7.9
				0-150	0.99	-0.40	0.996			1.18	-0.17	0.995		
1,8-Cineole	99.8	-	81	10-250	0.56	-0.13	0.999	5.2	-8.7	1.19	-0.54	0.996	3.5	-3.6
				0-150	0.52	-0.10	0.996			1.18	-0.75	0.985		
Linalool*	99.8	48 (R)-(-)/52 (S)-(+)	71	10-250	0.88	-0.72	0.994	8.6	-4.5	1.18	-0.88	0.996	3.1	-4.5
				0-150	0.86	-0.64	0.995			1.15	-0.10	0.999		
4-Terpineol	96.3	81 (S)-(+)/ 19 (R)-(-)	81	10-250	1.14	-0.87	0.997	7.2	-10.1	0.94	-0.30	0.997	4.1	-11.0
				0-150	1.13	-0.77	0.998			0.99	-0.24	0.995		
(*R*)-(+)-Borneol	84.7	90 (R)-(+)/10 (S)-(-)	95	10-250	2.43	-1.20	0.995	5.7	-13.4	1.03	-0.50	0.999	2.9	-13.4
				0-150	2.40	-0.83	0.996			1.02	-0.35	0.998		
Camphor	98.6	51 (R)/ 49 (S)	95	10-250	1.02	-0.63	0.996	6.3	-10.1	0.82	0.05	0.981	2.9	-11.9
				0-150	1.01	-0.44	0.996			0.82	0.04	0.985		
Lavandulol	82.6	95 (R)/5 (S)	69	10-250	0.23	-0.30	0.994	11.7	-17.0	0.90	-0.01	0.982	9.1	-18.4
				0-150	0.21	-0.14	0.988			0.91	-0.12	0.987		
linalyl acetate	99	50 (R)-(-)/50 (S)-(+)	93	10-250	0.93	-1.15	0.999	9.1	-6.5	0.89	-0.82	0.990	1.1	-2.1
				0-150	0.90	-0.80	0.996			0.87	-0.57	0.990		
(*R*)-(-)Lavandulol acetate	77	99 (R)-(-)	136	10-250	0.07	-0.10	0.994	9.8	-16.0	0.98	-0.78	0.998	5.1	12.7
				0-150	0.07	-0.07	0.989			0.90	-0.66	0.997		

Table 3

	***Mentha arvensis* L. (CS ARV)**			***Mentha x piperita* L. (CS PEPP)**			***Mentha x gentilis* (CS GENT)**			***Mentha spicata* L. (CS SPEAR)**		
	MS(Ti)	**FID**		**MS(Ti)**	**FID**		**MS(Ti)**	**FID**		**MS(Ti)**	**FID**	
Analyte	**g/100g**	**g/100g**	**Abs. Error %**	**g/100g**	**g/100g**	**Abs. Error %**	**g/100g**	**g/100g**	**Abs. Error %**	**g/100g**	**g/100g**	**Abs. Error %**
Limonene	1.63	1.73	6.1	1.46	1.58	7.5	5.16	5.35	3.5	7.56	7.46	1.3
1,8-Cineole	0.96	1.00	4.3	6.01	5.55	8.2	2.50	2.39	4.7	1.36	1.36	0.2
Linalool	0.14	0.13	11.1	0.34	0.32	7.4	0.06	0.06	7.6	0.19	0.18	8.9
Isopulegol	0.67	0.72	6.4	0.08	0.09	13.0	-	-	-	-	-	-
Menthone[a]	6.44	6.97	7.5	16.68	14.98	11.3	1.22	1.10	10.3	0.18	0.20	5.6
Menthofurane	-	-	-	2.16	2.05	5.3	-	-	-	-	-	-
Neomenthol	1.24	1.23	0.4	2.04	1.92	6.4	-	-	-	-	-	-
Menthol[b]	65.00	69.87	7.0	36.35	38.43	5.4	0.21	0.25	17.0	0.38	0.42	11.3
4-Terpineol	0.40	0.39	0.8	1.01	0.96	4.5	0.14	0.13	5.6	1.06	1.08	1.9
Isomenthol	0.51	0.55	6.0	1.33	1.29	3.8	-	-	-	-	-	-
Neoisomenthol	0.35	0.33	6.4	0.46	0.47	2.0	-	-	-	-	-	-
Pulegone	0.43	0.45	5.7	1.36	1.25	8.3	-	-	-	-	-	-
Carvone[b]	-	-	-	-	-	-	72.20	67.80	6.5	57.66	54.59	5.6
Menthyl acetate	2.55	2.28	11.9	5.62	5.28	6.6	0.06	0.06	4.5	0.07	0.07	2.0
Quality Control Analytes												
Decane	0.15	0.14	5.5	0.15	0.15	2.3	0.16	0.16	1.7	0.16	0.16	6.2
Undecane	0.15	0.15	0.6	0.15	0.15	0.5	0.15	0.15	0.5	0.16	0.16	1.3
Dodecane	0.15	0.16	5.0	0.16	0.15	7.1	0.16	0.16	0.2	0.15	0.16	5.1
Tridecane	0.15	0.16	6.9	0.16	0.16	1.1	0.15	0.16	6.8	0.15	0.15	3.5

	***Lavandula angustifolia* (CS LAV01)**			***Lavandula angustifolia* (CS LAV02)**			***Lavandula angustifolia* (CS LAV03)**			**Lavandin* (CS GROSS)**		
	MS(Ti)	**FID**		**MS(Ti)**	**FID**		**MS(Ti)**	**FID**		**MS(Ti)**	**FID**	
Analyte	**g/100g**	**g/100g**	**Abs. Error %**	**g/100g**	**g/100g**	**Abs. Error %**	**g/100g**	**g/100g**	**Abs. Error %**	**g/100g**	**g/100g**	**Abs. Error %**
1,8-Cineole[a]	3.32	3.46	4.0	4.57	4.04	13.2	8.98	10.07	10.8	5.94	7.08	16.0
(*S*)-(-)-Limonene	0.26	0.26	1.3	0.29	0.28	4.1	1.07	1.02	5.1	0.21	0.20	5.3
(*R*)-(+)-Limonene	0.72	0.66	9.1	0.90	0.79	13.9	1.19	1.10	8.5	1.27	1.17	8.7
(*S*)- (-)-Camphor	0.10	0.11	11.6	0.02	0.02	13.8	0.04	0.04	11.4	0.10	0.10	8.3
(*R*)- (+)-Camphor[a]	2.14	2.08	2.9	4.91	5.40	9.0	7.89	8.48	6.9	5.05	5.10	1.0
(*R*)-(-)-Linalool[b]	32.34	29.88	8.2	25.80	25.97	0.7	16.91	15.42	9.7	23.90	25.66	6.9
(*S*)-(+)-Linalool	2.96	2.88	2.8	1.92	1.67	15.0	2.20	1.99	10.3	2.13	2.01	6.0
(2*S*)- (+)-Borneol	0.30	0.26	15.4	0.07	0.06	17.3	0.15	0.13	14.4	0.07	0.06	9.6
(2*R*)- (-)-Borneol[a]	1.74	1.62	7.4	1.87	1.98	5.6	5.21	5.84	10.8	2.53	2.21	14.4
(*R*)-(-)-Linalyl acetate[b]	52.34	56.20	6.9	46.80	41.78	12.0	9.79	10.85	9.7	27.46	32.59	15.8
(*S*)-(+)-Linalyl acetate	0.88	0.80	10.0	0.26	0.25	7.3	0.27	0.26	3.1	0.88	0.80	10.3
(*S*)-(+)-4-Terpineol	2.93	2.58	13.7	1.51	1.69	10.4	0.07	0.06	11.5	2.35	2.55	7.9
(*R*)-(+)-4-Terpineol	0.13	0.12	9.0	0.09	0.09	7.3	0.33	0.30	10.1	0.21	0.18	12.9
(*R*)-(-)-Lavandulol acetate	1.24	1.18	5.1	2.12	1.80	18.2	1.24	1.14	8.9	2.16	2.07	4.2
(*R*)-(-)-Lavandulol	0.94	0.92	2.2	0.38	0.36	4.4	0.97	0.84	15.2	0.84	0.80	5.6
Quality Control Analytes												
Decane	0.15	0.14	5.5	0.15	0.15	2.3	0.16	0.16	1.7	0.16	0.16	6.2
Undecane	0.15	0.15	0.6	0.15	0.15	0.5	0.15	0.15	0.5	0.16	0.16	1.3
Dodecane	0.15	0.16	5.0	0.16	0.15	7.1	0.16	0.16	0.2	0.15	0.16	5.1

Tridecane	0.15	0.16	6.9	0.16	0.16	1.1	0.15	0.16	6.8	0.15	0.15	3.5

**: Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik
a: quantification was done on EO at 1 mg/L solution
b:quantification was done on EO at 500 µg/L solution

Table 4

							Mentha arvensis L. (CS ARV)			*Mentha x piperita* L. (CS PEPP)			*Mentha x gentilis* (CS GENT)			*Mentha spicata* L. (CS SPEAR)		
Analyte	**MW**	**Formula**	**^{1}D (min)**	**^{2}D (s)**	**I^T_S**	**$I^T_{S\,tab}{}^S$**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**
α-Thujene	136	C10H16	6.92	1.05	938	931	0.03	0.04		0.06	0.05		0.03	0.03		0.02	0.02	
α-Pinene	136	C10H16	7.08	1.09	942	939	0.47	0.71		0.84	0.79		0.31	0.27		0.49	0.45	
Sabinene	136	C10H16	8.58	1.25	978	976	0.18	0.27		0.07	0.08		0.01	0.01		0.30	0.28	
β-Pinene	136	C10H16	8.59	1.26	980	980	0.51	0.77		1.72	1.62		0.77	0.68		0.66	0.60	
Myrcene	136	C10H16	9.17	1.29	992	991	0.09	0.14		0.17	0.16		0.26	0.23		1.35	1.24	
3-Octanol	130	C8H18O	9.42	1.84	998	993	0.20	0.34		0.22	0.24		1.21	1.22		0.91	0.94	
α-Terpinene	136	C10H16	10.17	1.37	1016	1018	-	-		0.21	0.20			-			-	
p-Cymene	136	C10H16	10.50	1.46	1024	1026	0.02	0.02		0.20	0.17			-			-	
Limonene	136	C10H16	10.67	1.40	1028	1031	1.18	1.78	1.73	1.72	1.62	1.58	6.27	5.53	5.35	8.42	7.72	7.46
1,8-Cineole	154	C10H18O	10.75	1.62	1030	1033	0.63	1.08	1.00	5.89	6.32	5.55	2.68	2.68	2.39	1.50	1.50	1.36
cis-β-Ocimene	136	C10H16	11.08	1.38	1038	1040	-	-		0.18	0.17			-			-	
γ-Terpinene	136	C10H16	11.92	1.40	1058	1062	-	-		0.40	0.38			-			-	
cis-Sabinene hydrate	154	C10H18O	12.25	2.10	1066	1068	-	-		0.89	0.96		0.11	0.11		1.28	1.33	
α-Terpinolene	136	C10H16	13.17	1.45	1088	1088	-	-		0.12	0.11			-			-	
Linalool	154	C10H18O	13.75	2.07	1102	1100	0.06	0.15	0.13	0.34	0.37	0.32	0.07	0.07	0.06	0.20	0.20	0.18
3-Octyl acetate	172	C10H20O2	14.17	1.72	1111	1124	-	-		0.10	0.12			-		0.03	0.04	
Isopulegol	154	C10H18O	15.75	2.11	1145	1146	0.44	0.76	0.72	0.09	0.10	0.09	-	-	-	-	-	-
trans-Sabinol	152	C10H16O	15.75	2.3	1145	1142	-	-			-		0.08	0.09		0.05	0.05	
Menthone	154	C10H18O	16.17	2.57	1154	1154	4.93	8.47	6.97	17.03	18.26	14.98	1.30	1.31	1.10	0.19	0.19	0.20
Menthofuran	150	C10H14O	16.50	1.90	1162	1164	-	-	-	1.95	2.18	2.05	0	-	-	-	-	-
Isomenthone	154	C10H18O	16.58	2.47	1164	1164	2.98	5.12		4.86	5.21		0.24	0.24		0.04	0.04	
Neomenthol	156	C10H20O	16.67	2.25	1165	1165	0.88	1.36	1.23	2.02	2.13	1.92	0	-	-	-	-	-
Menthol	156	C10H20O	17.17	2.83	1176	1173	72.32	78.64	69.87	41.12	43.23	38.43	0.27	0.27	0.25	0.45	0.45	0.42
4-Terpineol	154	C10H18O	17.49	2.11	1183	1189	0.24	0.40	0.39	0.96	1.03	0.96	-	0.12	0.13	1.11	1.15	1.08
Isomenthol	156	C10H20O	17.50	2.22	1184	1182	0.27	0.45	0.55	1.07	1.13	1.29	-	-	-	-	-	-
α-Terpineol	154	C10H18O	17.75	2.63	1189	1191	-	-			-		0.01	0.42		0.15	0.16	
Neoisomenthol	156	C10H20O	17.75	2.29	1189	1188	0.22	0.37	0.33	0.51	0.53	0.47	-	-	-	-	-	-
Dihydrocarveol	154	C10H18O	18.08	2.47	1196	1192	-	-			-		0.93	0.93		1.96	2.03	
trans-Dihydrocarvone	152	C10H16O	18.42	2.45	1204	1200	-	-			-		0.12	0.12		0.20	0.21	
trans-Carveol	152	C10H16O	19.25	2.45	1223	1217	-	-			-		0.46	0.47		0.26	0.28	

cis-3-Hexenyl isovalerate	184	C11H20O2	19.92	1.85	1238	1240	0.22	0.41			-			-			-	
Pulegone	152	C10H16O	19.92	2.43	1238	1237	0.25	0.44	0.45	1.15	1.26	1.25	-	-	-	-	-	-
cis-Carveol	152	C10H16O	19.92	2.44	1238	*1242*[£]	-	-			-		0.55	0.56		0.28	0.30	
Carvone	150	C10H14O	20.42	3.19	1249	1242	-	-	-	0	-	-	72.66	72.82	67.80	67.22	72.68	54.59
Piperitone	152	C10H16O	20.58	2.72	1253	1252	0.26	0.46		0.47	0.51		0.22	0.22		0.14	0.14	
Neomenthyl acetate	198	C12H22O2	21.50	1.98	1273	1275	-	-		0.27	0.32			-			-	
trans-Carvone Oxide	166	C10H14O2	21.75	2.81	1279	1277	-	-			-		0.16	0.19		0.17	0.21	
Menthyl acetate	198	C12H22O2	22.42	2.11	1294	1294	1.42	2.65	2.28	5.27	6.11	5.28	0.04	0.07	0.06	0.07	0.08	0.07
Isomenthyl acetate	198	C12H22O2	23.17	2.07	1312	1306	-	-		0.02	0.02			-		0.07	0.08	
Dihydrocarvyl acetate	196	C12H20O2	23.92	2.08	1330	1325	-	-			-		0.54	0.58		0.44	0.49	
cis-Carvyl acetate	194	C12H18O2	25.33	2.1	1364	1362	-	-			-		0.15	0.17		0.41	0.48	
β-Bourbonene	204	C15H24	26.17	1.70	1384	1384	0.07	0.10		0.43	0.40		0.94	0.81		1.79	1.60	
β-Elemene	204	C15H24	26.50	1.73	1392	1391	0.03	0.04		0.19	0.17		0.19	0.17		0.19	0.17	
trans-β-Caryophyllene	204	C15H24	27.58	1.88	1418	1418	0.19	0.29		2.41	2.24		0.66	0.57		1.20	1.07	
α-Humulene	204	C15H24	29.00	1.84	1452	1454	-	-		0.11	0.10			-			-	
trans-β-Farnesene	204	C15H24	29.25	1.68	1458	1458	-	-		0.31	0.29		0.08	0.07		0.14	0.13	
Germacrene D	204	C15H24	30.17	1.93	1480	1480	0.07	0.11		2.12	1.96			-			-	
Bicyclogermacrene	204	C15H24	30.75	1.87	1494	1494	-	-		0.34	0.32			-			-	
δ-Cadinene	204	C15H24	31.83	1.73	1520	1524	0.02	0.04		0.08	0.07			-			-	
Spathulenol	220	C15H24O	33.92	2.42	1570	1576	0.02	0.04		0.05	0.05			-			-	
Caryophyllene oxide	220	C15H24O	34.08	2.44	1574	1581	0.04	0.06		0.12	0.12		0.50	0.48		0.19	0.19	
Viridiflorol	222	C15H26O	34.42	2.35	1582	1590	-	-		0.45	0.45			-			-	

$: R.P. Adams Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition Allured Publishing Corporation, 2007,Carol Stream US
£: Hognadottir, A., Rouseff, R.L. *J. Chromatogr. A.* **2003**, 998, 201

Table 5

							Lavandula angustifolia (CS LAV01)			*Lavandula angustifolia* (CS LAV02)			*Lavandula angustifolia* (CS LAV03)			Lavandin* (CS GROSS)		
Analyte	**MW**	**Formula**	**^{1}D (min)**	**^{2}D (s)**	**I^T_S**	**$I^T_{S\ tab}{}^S$**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**	**2D Volume %**	**g/100 g PRF**	**g/100 g Ext Cal**
α-Pinene	136	C10H16	8.42	1.07	930	924	0.17	0.19		0.42	0.42		0.75	0.80		0.44	0.49	
(*1S*,*4R*)-(-)-Camphene	136	C10H16	8.42	1.17	930	917	0.09	0.09		0.04	0.04		0.08	0.08		0.04	0.04	
(*1R*,*4S*)-(+)- Camphene	136	C10H16	9.00	1.14	945	934	0.07	0.08		0.28	0.28		0.47	0.50		0.22	0.25	
(*1R*)-(+)-β-Pinene	136	C10H16	9.58	1.25	960	947	0.07	0.07		0.18	0.18		0.31	0.34		0.28	0.30	
(*1S*)-(-)-β-Pinene	136	C10H16	10.00	1.24	968	957	0.07	0.07		0.15	0.14		0.27	0.29		0.20	0.22	
(*1R*,*5R*)-(+)-Sabinene	136	C10H16	10.75	1.20	982	973	0.03	0.04		0.08	0.08		0.16	0.17		0.12	0.13	
1,8-Cineole	154	C10H18O	11.08	1.72	989		2.65	3.28	2.70	4.00	4.50	3.59	9.16	11.12	8.39	6.14	7.69	5.90
Myrcene	136	C10H16	11.58	1.22	998		0.51	0.55		0.78	0.77		0.92	0.88		0.58	0.64	
δ-3-Carene	136	C10H16	13.25	1.20	1030		0.12	0.13		0.06	0.06		0.06	0.07		0.08	0.09	
1-Hexanol	102	C6H14O	13.75	0.92	1040	1042	0.09	0.13		0.07	0.09		0.13	0.20		0.08	0.12	
(*E*)-3-Hexen-1-ol	100	C6H12O	14.50	0.88	1054		0.07	0.10		-	-		1.92	2.59		0.04	0.06	
(-)-β-Phellandrene	136	C10H16	14.50	1.29	1054		0.13	0.14		0.21	0.20		0.04	0.04		0.02	0.02	
(*S*)-(-)-Limonene	136	C10H16	14.83	1.21	1060	1057	0.25	0.27	0.26	0.29	0.29	0.28	1.01	1.08	1.02	0.19	0.21	0.20
trans-β-Ocimene	136	C10H16	15.42	1.31	1071		1.75	1.91		1.01	1.00		0.50	2.17		0.32	0.35	
cis-β-Ocimene	136	C10H16	15.50	1.23	1073	1071	1.92	2.09		0.78	0.77		2.04	0.53		1.05	1.16	
(*R*)-(+)-Limonene	136	C10H16	15.58	1.17	1075	1072	0.51	0.56	0.53	0.99	0.98	0.93	1.19	1.27	1.20	0.94	1.04	0.98
(+)-*trans* Linalool oxide	170	C10H18O2	16.75	1.68	1097	1094	0.17	0.25		0.07	0.09		0.09	0.12		0.09	0.14	
(-)-*trans* Linalool oxide	170	C10H18O2	17.10	1.68	1101	1101	0.04	0.05		0.03	0.04		0.02	0.02		0.04	0.05	
(-)-*cis* Linalool oxide	170	C10H18O2	17.17	1.83	1105	1101	0.15	0.22		0.04	0.05		0.07	0.10		0.07	0.10	
trans-Sabinene hydrate	154	C10H18O	17.42	1.66	1109		0.04	0.05		0.05	0.05		0.05	0.12		0.10	0.12	
Octen-1-ol acetate	170	C10H18O2	17.42	1.80	1109	1105	0.43	0.61		0.30	0.39		0.10	0.06		0.27	0.38	
1-Octen-3-ol	128	C8H16O	18.25	1.10	1125		0.24	0.31		0.08	0.09		0.47	0.58		0.13	0.17	
(*S*)- (-)-Camphor	152	C10H16O	19.25	2.37	1143	1136	0.08	0.10	0.09	0.01	0.02	0.02	0.02	0.03	0.03	0.06	0.08	0.08
Hexyl propanoate	158	C9H18O	19.33	1.57	1145		0.19	0.26		0.12	0.15		0.20	0.27		0.12	0.17	
(*R*)- (-)-Camphor	152	C10H16O	19.58	2.41	1149	1142	1.55	1.95	1.91	5.60	6.43	6.33	8.36	10.35	10.21	4.49	5.73	5.63
(*R*)-(-)-Linalool	154	C10H18O	20.75	1.76	1171	1169	27.93	34.58	24.90	27.13	30.52	22.05	33.33	17.96	13.26	24.06	30.10	21.76
(*S*)-(+)-Linalool	154	C10H18O	21.83	1.52	1191		1.87	2.32	2.15	1.45	1.63	1.65	1.51	1.83	1.79	1.57	1.97	1.89
(2*S*)- (+)-Borneol	154	C10H18O	22.50	2.18	1203	1195	0.23	0.28	0.23	0.05	0.06	0.05	0.13	0.16	0.13	0.07	0.09	0.07
(2R)- (-)-Borneol	154	C10H18O	22.83	2.20	1209	1202	1.11	1.38	1.29	2.02	2.27	2.01	6.19	7.52	6.26	1.96	2.45	2.16
Hexyl butanoate	172	C10H20O2	23.50	1.77	1220	1215	0.28	0.39		0.58	0.73		0.37	0.51		0.33	0.47	
(*R*)-(-)-Linalyl acetate	196	C12H20O2	24.58	2.66	1239	1232	32.01	43.64	40.50	15.80	37.18	34.59	7.60	10.16	9.85	26.24	36.15	33.64
(*S*)-(+)-Linalyl acetate	196	C12H20O2	24.92	2.24	1245	1240	0.61	0.83	0.71	0.24	0.29	0.25	0.06	0.29	0.25	0.39	0.54	0.46
(*S*)-(+)-4-Terpineol	154	C10H18O	25.17	1.74	1249	1247	1.80	2.23	2.12	1.56	1.76	1.70	2.59	0.07	0.06	2.39	2.99	2.79
Hexyl-2-methyl butyrate	186	C11H22O2	25.17	1.92	1249		0.06	0.08		0.13	0.16		0.22	3.43		0.08	0.11	
(*R*)-(-)-4-Terpineol	154	C10H18O	25.50	1.64	1255	1253	0.09	0.11	0.10	0.08	0.09	0.08	0.21	0.26	0.22	0.13	0.17	0.15
Hexyl Isovalerate	184	C11H20O2	25.58	1.90	1256		0.06	0.08		0.13	0.16		0.10	0.13		0.17	0.24	
(*R*)-(-)-Lavandulyl acetate	196	C12H20O2	26.33	2.52	1269	1263	0.82	1.11	0.93	1.77	2.20	2.08	0.82	1.10	0.91	2.15	2.97	2.67
(*R*)-(-)-Lavandulol	154	C10H18O	26.58	1.57	1274	1275	0.73	0.90	0.82	0.30	0.34	0.31	0.56	0.68	0.62	0.54	0.68	0.62
(*S*)-(-)-α-Terpineol	154	C10H18O	27.92	1.63	1297	1297	0.29	0.36		0.19	0.21		0.25	0.31		0.27	0.34	
(*R*)-(+)-α-Terpineol	154	C10H18O	28.58	1.58	1309	1310	0.81	1.00		0.56	0.64		0.71	0.87		0.73	0.91	
Hexyl Tiglate	184	C11H20O2	31.42	2.05	1362	1359	0.07	0.09		0.22	0.27		0.08	0.11		0.15	0.21	
Geraniol	154	C10H18O	31.67	1.74	1367	1368	0.51	0.63		0.29	0.32		0.30	0.36		0.35	0.44	
Neryl Acetate	196	C12H20O2	32.17	2.31	1376	1372	0.42	0.57		0.24	0.30		0.18	0.24		0.25	0.34	
α-Santalene	204	C15H24	33.33	2.16	1398	1391	0.43	0.46		0.11	0.10		0.20	0.21		0.21	0.22	
(-)-*trans* Caryophyllene	204	C15H24	34.00	2.28	1410	1405	1.81	1.94		1.98	1.93		1.39	0.95		1.53	1.66	

Geranyl acetate	196	C12H20O2	34.17	2.20	1413	1411	1.29	1.75	0.02	0.03	0.04	0.65	0.55	0.75
trans-ß-Farnesene	204	C15H24	36.50	2.13	1457	1454	0.88	0.94	0.94	0.91	1.02	1.07	1.17	1.27
Germacrene D	204	C15H24	37.17	2.42	1469	1464	0.17	0.18	0.47	0.45	0.41	0.43	0.58	0.62
Lavandulyl isovalerate	238	C15H26O2	38.42	2.63	1492	1489	0.08	0.11	0.23	0.27	0.31	0.39	0.26	0.34
ß-Bisabolene	204	C15H24	39.42	2.13	1511	1509	0.06	0.07	0.09	0.09	0.07	0.07	0.12	0.13
α-Bisabolol	222	C15H26O	50.08	2.67	1719	1724	0.06	0.08	0.56	0.59	0.31	0.35	0.27	0.32

Ş: E. Liberto, C. Cagliero, B. Sgorbini, C. Bicchi, D. Sciarrone, B.D. Zellner, L. Mondello, P. Rubiolo, *J. Chromatogr. A.* **2008**, 1195, 117.

Table 6

Analyte	Enantiomeric Composition % (EC%)			
	CS LAV01	**CS LAV02**	**CS LAV03**	**CS GROSS**
(R)-(-)-Linalool	85.6	94.9	90.7	93.9
(S)-(+)-Linalool	14.4	5.1	9.3	6.1
(R)-(-)-Linalyl acetate	98.1	99.2	97.2	98.6
(S)-(+)-Linalyl acetate	1.9	0.8	2.8	1.45

Figure 1

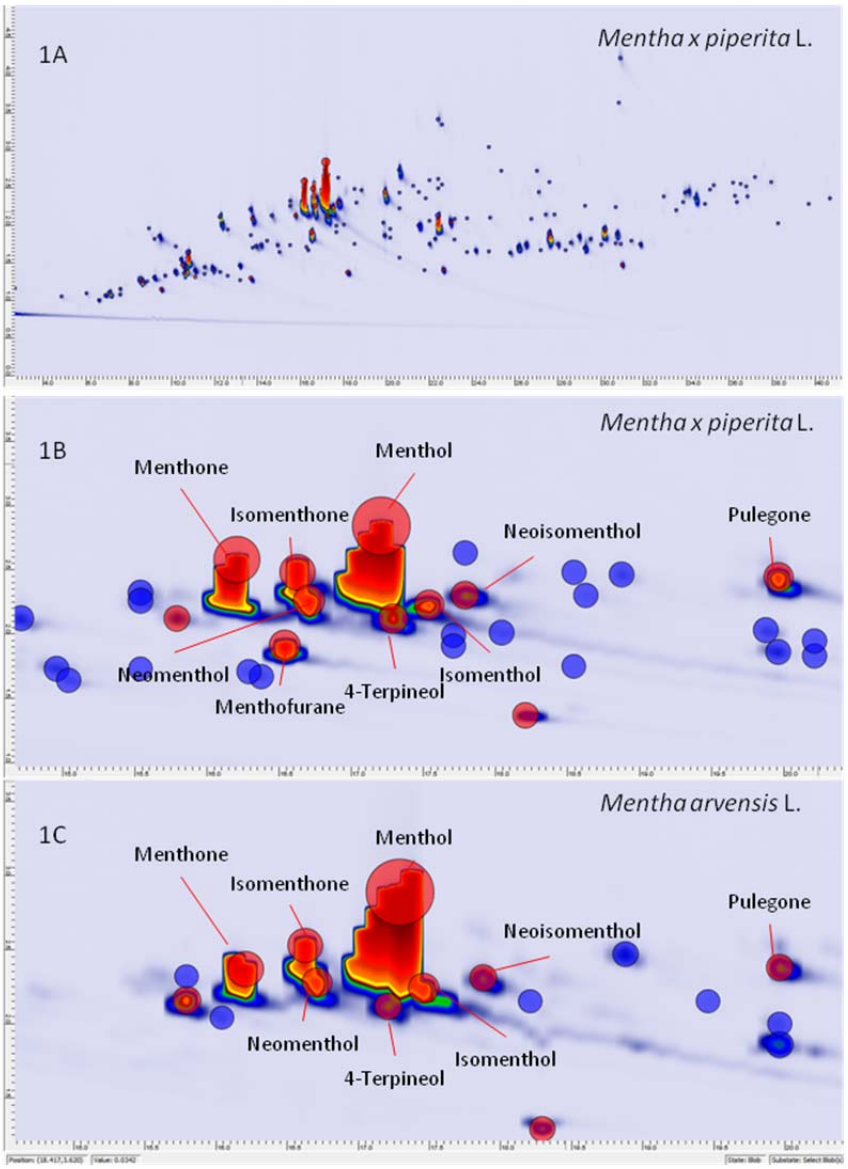

1A
Mentha x piperita L.
1B
Mentha x piperita L.
Menthone
Menthol
Isomenthone
Neoisomenthol
Pulegone
Neomenthol
Menthofurane
4-Terpineol
Isomenthol
1C
Mentha arvensis L.
Menthone
Menthol
Isomenthone
Pulegone
Neoisomenthol
Neomenthol
4-Terpineol
Isomenthol

Figure 2

2A

2B

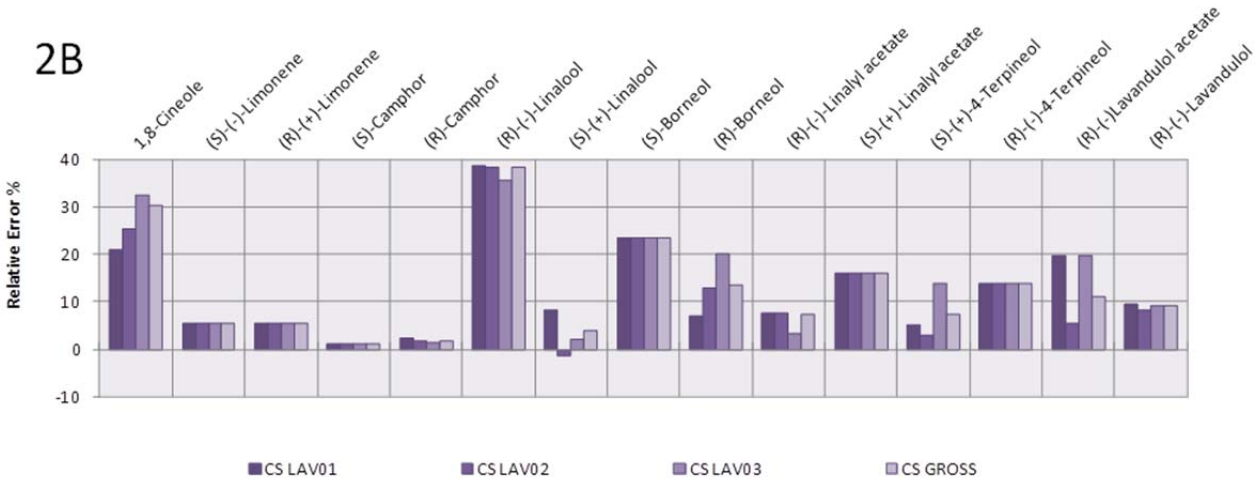
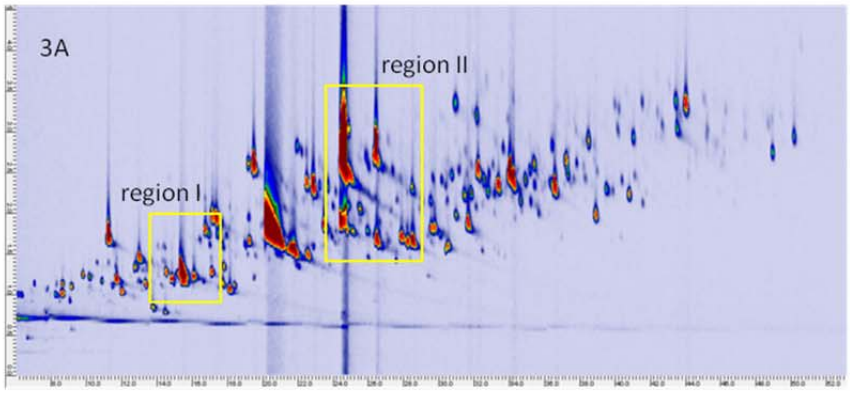
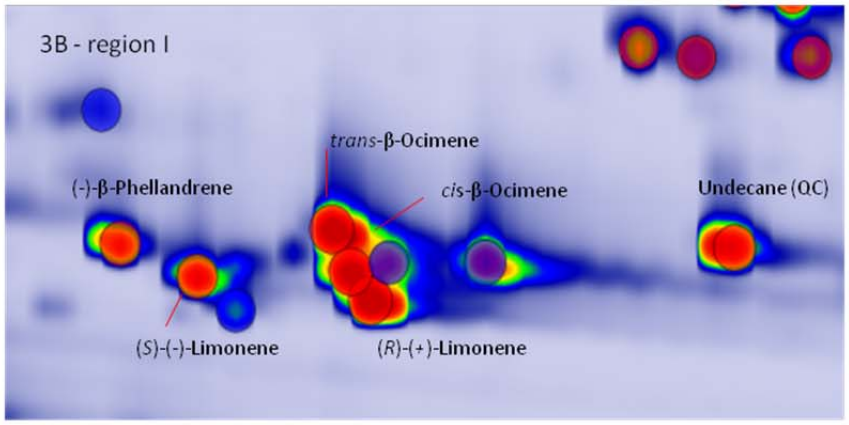
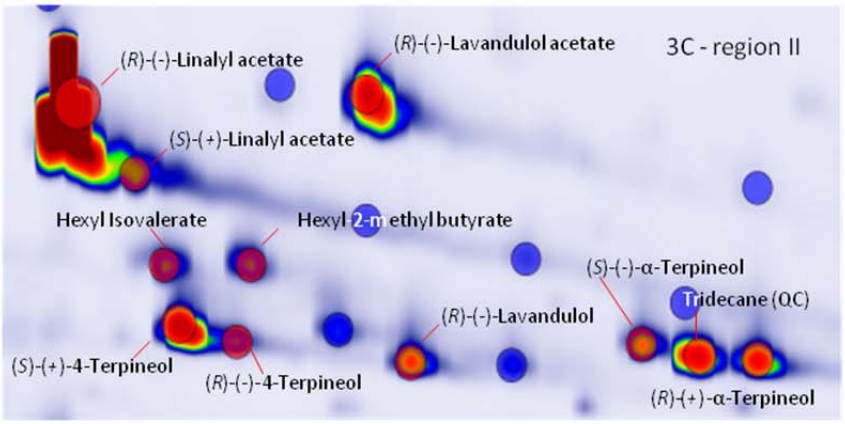


Figure 3


3A
region II
region I


3B - region I
trans-β-Ocimene
(-)-β-Phellandrene
cis-β-Ocimene
Undecane (QC)
(S)-(-)-Limonene
(R)-(+)-Limonene


(R)-(-)-Linalyl acetate
(R)-(-)-Lavandulol acetate
3C - region II
(S)-(+)-Linalyl acetate
Hexyl Isovalerate
Hexyl 2-methylbutyrate
(S)-(-)-α-Terpineol
Tridecane (QC)
(R)-(-)-Lavandulol
(S)-(+)-4-Terpineol
(R)-(-)-4-Terpineol
(R)-(+)-α-Terpineol

Supplementary Figure 1