Optimization of GC-MS Method for Analysis of Basil Essential Oils from Algeria: Design of Experiments and Exploratory Pattern Recognition

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An optimization procedure for multistep temperature-programmed capillary gas chromatography was developed for the analysis of basil essential oil. The current study was planned after performing the following three steps: a preliminary chromatographic study, primary parameter screening, and final method optimization by using 2⁵ factorial and Doehlert designs (DOE). The optimized conditions were obtained by using a split ratio of 1/2, a gas flow rate of 1.3 mL/min, and a heating rate of 2°C/min. This set of conditions was later used for the optimization of the final method. The optimal experimental conditions were subsequently obtained by response surface optimization (isotherm duration = 6.2 min and slope = 1.06°C/min). The compositions of essential oils from six varieties of basil (Ocimum B. (1), Ocimum B. purpureascens opal (2), Ocimum B. minimum (3), Ocimum. B. cinnamon (4), Ocimum B. (marcellas) (5), and Ocimum B. Citriodora (6)) grown in four regions of Algeria (Ouargla, south of Algeria (O); Mostaganem, west of Algeria (M); Algiers, north of Algeria (A), and Constantine, east of Algeria (C)) were determined. A statistical study was performed based on hierarchical ascending classification and principal component analysis to elucidate the relationships between the basil varieties, their region of growth, and their essential oil composition. In addition to identifying several chemotypes, such as linalool, linalool/eugenol, linalool acetate, methyl eugenol, methyl chavicol, eugenol, methyl cinnamate E, and geraniol, a new compound, namely, octadecenamide (Z) (oleamide), was detected in the essential oil of Ocimum basilicum L. from Algiers (A1).

1. Introduction

Because of the richness of their composition, essential oils are difficult to analyse, and there is a need to develop new analytical methods by using various optimization techniques. The design of experiments (DOEs) refers to a set of multivariate mathematical and statistical techniques. The combination of the response surface methodology (RSM) and DOE is a powerful data collection and analysis tool which is used in various experimental situations. It allows for the manipulation of multiple input factors and the determination of their effects on the desired output (response). By allowing one to change multiple inputs simultaneously, DOE helps identify any significant interactions that may be missed when varying only one factor at a time.

Ocimum basilicum is an aromatic plant rich in active ingredients and is being studied by researchers around the world with the aim of identifying the composition of its extracts, evaluating their antioxidant, antibacterial, and antifungal activities [1–8], and elucidating the relationship between the composition of its extracts and their activities [9–11].
The use of statistical analysis for determining the composition of essential oils by using techniques such as hierarchical ascending classification (HAC) and principal component analysis (PCA) has been shown to be a reliable and powerful approach for evaluating several essential oils simultaneously based on their similarities.

To optimize the analysis of essential oils of O. basilicum by using gas chromatography coupled with mass spectrometry, we used the DOE approach. The optimized model was then used to analyze 24 basil essential oils from six varieties grown in four different regions of Algeria.

2. Materials and Methods

2.1. Plant Materials. The plants were grown in four regions of Algeria at the same time: Ouargla, south of Algeria (O); Mostaganem, west of Algeria (M); Algiers, north of Algeria (A), and Constantine, east of Algeria (C). The essential oil of O. basilicum L. from Constantine (C) was used for optimization. Table 1 summarizes the geographical data for the four study regions.

2.2. Extraction of Essential Oils. The essential oils were extracted from aerial parts and dried under amber by steam distillation using a Clevenger apparatus. The obtained essential oil samples were stored at 4°C until analysis.

2.3. GC-FID and GC-MS Conditions. All the optimization experiments were performed by using a GCMS-QP2010 gas chromatography-mass spectrometry (GC-MS) system with an AOC-20i autosampler (Shimadzu, Japan) and a Rxi-1MS GC column (30 m × 0.25 mm id × 0.25 µm film thickness) (100% dimethyl polysiloxane) (Restek, USA). The maximum temperature was 330°C, and the mass range was 45–500 g. The carrier gas (He) flow rate and split ratio were not optimized during the first part of the study. The test samples were diluted to a fixed concentration by using dichloromethane in a volume of 1 µL.

The composition of the 24 essential oils of the various O. basilicum L. varieties was identified by using a Shimadzu GC-2010 chromatograph (employed for the GC-FID analysis) equipped with an Rxi-5 ms capillary column (30 m × 0.25 mm, film thickness of 0.25 µm). He was used as the carrier gas at a flow rate of 1.44 mL/min. The oven temperature was maintained at 45°C for 10 min, increased to 180°C at a rate of 3°C/min, maintained at 180°C for 5 min, then increased to 280°C at a rate of 5°C/min and 330°C at a rate of 10°C/min for 2 min. An electron ionization system with an ionization energy of 70 eV was used for the GC-MS analysis. The injector temperature was set to 330°C. The samples diluted in 1 µL of dichloromethane were injected in the split mode (30:1).

The essential oil components issued from the capillary column were identified based on a comparison of their retention index values (KI, calculated from the GC-FID analysis) with those reported in literature [12], as well as by comparing their mass fragmentation patterns with those in existing databases. Quantification was performed by calculating the relative areas with respect to the total area of the respective chromatograms by using the software LabSolutions (Shimadzu Instruments). We assumed that the response coefficient for all the compounds was equal to one. The percentage concentration (m/m) of each compound was expressed relative to that of all the constituents.

2.4. Experimental Design for Optimization. Two analyses were performed by using the software Minitab 18 to understand the results.

2.4.1. Preliminary GC-MS Study. A preliminary study was performed on the samples to determine the possible interferences that could have occurred during the temperature-programmed analysis. Figure 1 shows the chromatogram obtained by using the following temperature program: 45 to 180°C at 8°C/min and 180 to 330°C at 10°C/min. It is also important to mention that a split ratio of 1/30 and gas flow rate of 1 mL/min were used for the study.

The chromatogram shows only one possible interference, which occurs between eucalyptol and D-limonene. These were considered the main targets for separation in the first part of the study. It should be noted that the existence of only one interference does not mean that all the compounds in the samples were well separated. It is likely that the overlapping of peaks would occur, especially in the case of compounds with similar ebullition temperatures.

2.4.2. First Study. A 2^3 factorial design was employed to study the effects of the various variables on the chromatographic response. In this design, the levels of the factors were varied independently, with each factor having two levels (−1 and +1). This made it possible to perform optimization as long as the “weight” of the gas flow rate, split ratio, and slope, then rank the factors in order of importance based on the Pareto principle.

A 2^3 factorial design requires eight experiments. Thus, three centre points were included to explore the curvature as well as to estimate the repeatability of the experiment, resulting in a total of 11 experiments.

Random order of experience was followed to minimize the effect of potential errors. Each response was approximated by using a first-order polynomial regression function:
\[ Y_i = A_0 + A_1 X_1 + A_2 X_2 + A_3 X_3 + A_{12} X_1 X_2 + A_{13} X_1 X_3 + A_{23} X_2 X_3, \]

where \( Y \) is the response (two responses), \( i = 1 \) is the number of picks obtained from the analysis, and \( i = 2 \) is the experimental retention (resolution). In addition, \( X_1, X_2, \) and \( X_3 \) are the three investigated factors expressed as coded variables; \( A_0 \) is the global coefficient of the regression model; \( A_1 \) is the coefficient of slope factor \( X_1; A_2 \) is the coefficient of the gas flow rate factor, that is, \( X_2; A_3 \) is the coefficient of the split ratio factor, that is, \( X_3; A_{12} \) is the coefficient of interaction \( X_1 X_2; A_{13} \) is the coefficient of interaction \( X_1 X_3; \) and \( A_{23} \) is the coefficient of interaction \( X_2 X_3. \)

2.4.3. Second Study. Based on the results of the primary parameter screening process, the split ratio and gas flow rate were set to their optimal values, and the temperature was varied over a wide range to determine the optimal space. Subsequently, in the second study, the temperature was varied along with the other factors over a narrow range to determine the optimal temperature program for this analysis.

A decrease in the temperature resulted in the appearance of three more compound pairs (Table 2); their separation was the main objective of the second study.

Three factors were studied: the initial isotherm duration (ID), the slope of the temperature profile curve (S), and the final temperature (FT). The isotherm duration corresponding to the final temperature was not considered a factor. However, it was kept long in order to elute the compounds in the sample.

It should be noted that the choice of the range of the three parameters to optimize was based on previous analyses of basil essential oils by using this type of column.

To determine the optimal conditions for analysing the four compound pairs listed in Table 2, we selected the experimental design matrix proposed by the DOE. All the experiments were performed arbitrarily to ensure the independence of the responses. In addition, the central point of the experimental domain was repeated five times to account for all the possible errors during the analysis.

This type of matrix is characterized by its high efficiency, as different factors can be studied at different levels [13, 14]. Factors that deserve more attention are assigned more levels. In this study, the ID factor was described using three levels and the S factor using seven levels because the latter was the most critical parameter to adjust.

Table 1: Geographical data of four study regions.

<table>
<thead>
<tr>
<th></th>
<th>Ouargla (O)</th>
<th>Mostaganem (M)</th>
<th>Alger (A)</th>
<th>Constantine (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude</td>
<td>31°97'</td>
<td>35°94'</td>
<td>36°58'</td>
<td>36°36'</td>
</tr>
<tr>
<td>Longitude</td>
<td>5°34'</td>
<td>0°09'</td>
<td>2°90'</td>
<td>6°60'</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>128</td>
<td>55</td>
<td>270</td>
<td>944</td>
</tr>
</tbody>
</table>

Table 2: Compound pairs whose separation was optimized.

<table>
<thead>
<tr>
<th>Compound pairs</th>
<th>Interference code</th>
<th>Response, ( Y_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabinene/β-pinene</td>
<td>1</td>
<td>( Y_1 )</td>
</tr>
<tr>
<td>Eucalyptol/D-limonene</td>
<td>2</td>
<td>( Y_2 )</td>
</tr>
<tr>
<td>Geraniol/linalyl acetate</td>
<td>3</td>
<td>( Y_3 )</td>
</tr>
<tr>
<td>Eugenol/α-terpinyl acetate</td>
<td>4</td>
<td>( Y_4 )</td>
</tr>
</tbody>
</table>

Figure 1: GC-MS chromatogram of basil essential oil sample before optimization (1-eucalyptol/D-limonene).
Table 3: Experimental design and method parameters used for the first two studies.

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>First study</th>
<th>Second study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors/responses</td>
<td>2&lt;sup&gt;3&lt;/sup&gt; factorial design</td>
<td>Doehlert design</td>
</tr>
<tr>
<td>Number of runs</td>
<td>3/2</td>
<td>3/4</td>
</tr>
<tr>
<td>Method parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial temperature</td>
<td>45°C</td>
<td>18°C</td>
</tr>
<tr>
<td>Isotherm duration</td>
<td>5 min</td>
<td>5 and 10 min</td>
</tr>
<tr>
<td>Slope</td>
<td>2 and 6°C/min</td>
<td>1 and 2°C/min</td>
</tr>
<tr>
<td>Final temperature</td>
<td>180°C</td>
<td>80 and 180°C</td>
</tr>
<tr>
<td>Isothermal step at FT</td>
<td>10 minutes</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Split ratio</td>
<td>3/7 and 3/7</td>
<td></td>
</tr>
<tr>
<td>Gas flow rate</td>
<td>0.6 and 1.3 mL/min</td>
<td>1.3 mL/min</td>
</tr>
</tbody>
</table>

* Method parameters in bold were varied during respective stages, and the rest were kept constant.

The three factors \((X_1, X_2, \text{and} \ X_3)\) and the second-degree polynomial model used are described by

\[
Y_i = A_0 + A_1 X_1 + A_2 X_2 + A_3 X_3 + A_{11} X_1^2 + A_{22} X_2^2
+ A_{33} X_3^2 + A_{12} X_1 X_2 + A_{13} X_1 X_3 + A_{23} X_2 X_3,
\]

where \(Y_i\) is experimental resolution and is defined by

\[
Y_i = \frac{2(t_2 - t_1)}{\delta_2 + \delta_1}
\]

where \(t_i\) is the retention time for eluted compounds 1 and 2 and \(\delta_i\) is the peak width for these compounds.

Table 3 lists the experimental design and method parameters for the two studies, while Figure 2 shows the GC-MS optimization strategy based on the DOE.

2.5. Statistical Analysis of Compositions of Essential Oils. Based on the analytical results, once the number of individuals was sufficient, we performed a statistical study by using the software programs SYSTAT-13 and XLSTAT.

Specifically, we performed the following studies:

1. Analysis by hierarchical group or cluster tree (dendrogram), which allowed us to graphically highlight the similarities and relationships between the individual components

2. Principal component analysis (PCA), which is an extremely powerful tool for analysing large amounts of quantitative data. PCA is a factorial analysis technique in the sense that it produces factors (or principal axes), which are linear combinations of the initial variables and hierarchical and independent of each other

These analyses were performed to determine the groups that may recognise chemotypes. The concentrations of the primary compounds (>1%) in each sample studied were used for the statistical calculations. All the data should thus allow or not show the similarity of the composition of the essential oils of several populations and this, according to their geographical origin.

3. Results and Discussion

3.1. Optimization

3.1.1. First Study. The experimental plans and their corresponding responses are listed in Table 3. To determine the significance and validity of the proposed model, the regression model for each considered response was evaluated by using analysis of variance (ANOVA), and the response surface plots were generated by using the software Minitab. The equation model for the constants as well as the regression coefficients and statistical parameters for each response variable (i.e., the experimental resolution for the pairs of compounds and the number of peaks) is given in Table 4. As shown in Figures 2(a)–2(f), we could obtain three-dimensional (3D) diagrams by plotting the response against two of the factors while keeping the third factor constant (0). Based on the response graphs, the following conclusions could be made.

1. Effects of Variables on the Number of Peaks. The ANOVA results for the obtained responses showed that the model was significant (i.e., the \(p\) values were less than 0.05) for all three factors. It can be seen from equation (4) that

\[
NP = 113.88 + 15.63X_1 + 17.99X_2 + 16.38X_3
- 0.37X_1X_2 + 3.12X_1X_3 + 1.87X_2X_3.
\]

The number of peaks increased with an increase in all the operating variables, that is, the slope \((X_1)\), gas flow rate \((X_2)\), and split ratio \((X_3)\), which had the greatest effect on the first response. All the interactions were insignificant, and the previous model could be adjusted as follows:

\[
NP = 116 + 17.75X_1 + 15.87X_2 + 18.5X_3.
\]

The variance values of S = 5.988, \(r^2 = 96.75\%\), and Adj. \(r^2 = 94.58\%\) indicated good predictability.

2. Effects of Variables on Experimental Resolution. The ANOVA results for the obtained responses showed that the model was significant (the \(p\) value was less than 0.05) only in the case of the slope. By varying the system variables, the following regression equation could be obtained for predicting the experimental retention:
ER = 0.3710 – 0.2870X_1 + 0.0585X_2 – 0.0780X_3 
+ 0.0255X_1X_2 – 0.0060X_1X_3 – 0.0505X_2X_3. \hspace{1cm} (6)

The fact the regression coefficient for $X_1$ was negative and significant indicated good resolution between the compounds, which can be obtained with the simultaneous decrease of the slope and split ratio; the increase in the gas flow rate that turned out to be worthy of attention once the model was adjusted, all insignificant interactions were removed, and the interaction between the gas flow rate and split ratio was maintained in the equation to adjust the model despite its little importance:

Figure 2: 3D surface plots of response showing variations in response: (a–c) number of peaks and (d–f) experimental resolution as functions of slope (°C/min), gas flow rate (mL/min), and split ratio.
Based on the above-described results, it was concluded that the initial model could be reduced to a final four-parameter model of the following form:

\[ Y_1 = 0.644 - 0.09021S - 0.01633ID - 0.0319FT^2. \]  

(8)

As per the ANOVA results, for a 95% confidence level, the regression was statistically significant because the calculated F value (65.72) was greater than the critical F value (3.34). The prediction capability of Equation (8) was evaluated statistically, and it was found that 88.06% of the variability of \( Y_1 \) could be explained by this reduced model.

Variable \( Y_2 \) was also evaluated in a similar manner. The best mathematical approximation of the observed experimental retention was expressed using a reduced six-coefficient model:

\[ Y_2 = 0.89671 - 0.08732S - 0.02654ID - 0.0368S^2 \]

\[-0.284FT^2 + 0.0490FT \ast ID. \]

(9)

For the fitted model, the calculated F value (34.60) was greater than the critical F value (3.11) at the 95% confidence level (Table 7). This explained 78.41% of the experimental variability of \( Y_2 \).

Various polynomial models were evaluated for the third response, and the final function showed a clear dependence on the slope factor.

\[ Y_3 = 0.85579 - 0.11403S - 0.02316FT^2 - 0.02289ID^2. \]

(10)

The experimental F-test, with 3 and 14 degrees of freedom (F3/14 = 133.26) resulted in a value greater than the critical F value (3.34), thus confirming that Equation (10) was adequate. This model also explained 94.96% of the experimental variability of \( Y_3 \).

The term model Equation (2) was inadequate for describing the behaviour of the three factors investigated in this study. Therefore, it was reduced to a four-coefficient model after evaluating its goodness of fit. The simplified model can be described by the following equation:

\[ Y_4 = 0.25595 - 0.09454S - 0.02714FT^2 + 0.03786S^2. \]

(11)

The experimental F-test confirmed the adequacy of Equation (11) (F (87.98) > critical F (3.34)) at a 95% confidence level (see Table 8). This also explained 91.59% of the experimental variability of \( Y_2 \).
We confirmed that the optimal conditions listed in Table 6 allow for the accurate separation of the four pairs of compounds listed in Table 2. The chromatogram obtained under the optimal conditions is presented in Figure 6.

The predicted and experimental resolutions for the 12 interferences under the optimal conditions are listed in Table 7. Based on the four responses, it can be concluded that there was good agreement between the experimental and predicted values.

3.2. Statistical Analysis of Compositions of 24 Essential Oils from Ocimum basilicum. L. The ascending hierarchical classification (cluster tree) of the 24 samples analysed allowed us to detect the presence of three groups (Figure 7). Predominant group: the linalool group contains 11 essential oils (O1, M1, M2, O3, M4, C5, O5, O4, C4, M5 and M4), which are green; phenylpropanoid group: this group (eugenol/methyl eugenol/methyl chavicol/methyl cinnamate)
(a) Figure 5: Continued.
contains nine essential oils (C3, M3, A2, A1, C2, A4, O2, A5, and C1, which are red); and geranial group: this group contains four essential oils, namely, those of lemon basil (A6, C6, M6, and O6, which are orange).

In the cluster tree, distances smaller than 2 indicate highly similar or even identical compositions. Moreover, the similarity decreases slightly for distances in the 2–5 range and becomes minimal for distances in the 5–10 range.

The PCA results allowed the different directions of the three groups (individual components and variables) (Figures 8 and 9, respectively) to be observed more clearly. As the compositions of essential oils are highly complex, a difference in a single compound can cause a change in the orientation (angle) or distance (length of the axis), making the identification of the groups difficult.

For the first time, oleamide was identified in a concentration of 14.65% in the essential oil of a variety of Ocimum basilicum L. grown in Algiers (A1). Figure 10 shows the chromatogram of the essential oil of A1, while Figure 11 shows the experimental mass spectrum of oleamide as well as that taken from the NIST database.

The various varieties of Ocimum basilicum L. grown in the four different regions showed considerable diversity in their compositions, which can be explained by the differences in their geographical locations, properties of the water used for irrigation, and effects of the neighbouring crops.

Statistical analyses performed by using HAC and the PCA method allowed us to draw the following conclusions.

### 3.2.1. Ocimum basilicum L.
1. The essential oils of the samples from Ouargla and Mostaganem are similar in composition to those of a hybrid chemotype (linalool/eugenol).
2. A new compound, namely, octadecenamide (Z) (oleamide), was detected in the essential oil from Algiers. It is an active ingredient used in pharmacognosy and can induce sleep.
3. The essential oil from Constantine yielded a new chemotype, linalool acetate, at a rate of 40.09%.

### 3.2.2. Ocimum basilicum L. Purpurascens Cens.
1. The essential oils closest to their constituents were those from Ouargla and Mostaganem, with linalool being the chemotype.
2. The essential oil from Algiers contained methyl eugenol as a chemotype.
3. The essential oil from Constantine contained a methyl chavicol chemotype.

### 3.2.3. Ocimum basilicum L. Minimum.
1. The four essential oils showed significant differences in their constitutions.
2. The essential oil from Algiers contained the same chemotype.
as the essential oil from Mostaganem (methyl eugenol). (3) The essential oil from Ouargla contained linalool as a chemotype, while that from Constantine contained eugenol as a chemotype.

3.2.4. Ocimum basilicum L. Cinnamon. Two groups were distinguished: (1) the first group, which included the essential oils from Ouargla, Mostaganem, and Constantine, contained a methyl cinnamate (E) chemotype. (2) The second group was limited to the essential oil from Algiers, which contained a linalool chemotype.

3.2.5. Ocimum basilicum L. Marseillais. (1) Despite the differences in the constitutions of the four essential oils, they were considered to belong to the same group. This is because

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Table 8: Results of the validation of four statistical models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation (8)</th>
<th>Equation (9)</th>
<th>Equation (10)</th>
<th>Equation (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance</td>
<td>0.0133725 (17)</td>
<td>0.0148353 (17)</td>
<td>0.0115758 (17)</td>
<td>0.0122119 (17)</td>
</tr>
<tr>
<td>Pure error variance</td>
<td>0.000533 (5)</td>
<td>0.000483 (5)</td>
<td>0.001083 (5)</td>
<td>0.000933 (5)</td>
</tr>
<tr>
<td>Lack-of-fit variance</td>
<td>0.001970 (9)</td>
<td>0.002158 (7)</td>
<td>0.000793 (9)</td>
<td>0.001155 (9)</td>
</tr>
<tr>
<td>F_calculated</td>
<td>65.72</td>
<td>34.60</td>
<td>133.26</td>
<td>87.98</td>
</tr>
<tr>
<td>F_optimized</td>
<td>3.34</td>
<td>3.11</td>
<td>3.34</td>
<td>3.34</td>
</tr>
<tr>
<td>r(^2)</td>
<td>93.37%</td>
<td>93.51%</td>
<td>96.62%</td>
<td>94.96%</td>
</tr>
</tbody>
</table>

Degrees of freedom are listed within parentheses.

Figure 6: GC-MS chromatogram of basil essential oil sample obtained under optimal conditions. Slope = 1.06°C/min, final temperature = 121.41°C, and isotherm duration = 6.2 min. From left to right, (1) sabinene/β-pinene), (2) eucalyptol/D-limonene), (3) geraniol/linalyl acetate), and (4) eugenol/α-terpinyl acetate).

Figure 7: Cluster tree of 24 essential oils studied.
they all had the same chemotype, linalool. (2) Based on this result, it can be concluded that linalool is representative of the variety *Ocimum basilicum* L. Marseillais.

### 3.2.6. *Ocimum basilicum* L. Citriodora

(1) The two essential oils, namely, those from Mostaganem and Constantine, which are similar to that from Algiers, constitute a group that contains geraniol as the main product. (2) The essential oil from Ouargla contains linalool as the main compound.

Researchers worldwide are attempting to determine the composition of basil essential oils [15]. Wyller et al. reviewed the existing literature on the composition and biological activities of basil essential oils. Several active ingredients have been identified worldwide, depending on the geographical origin of basil. Shatar et al. used the seeds of *Ocimum basilicum* L. from the United States and found that the oxygenated constituents, namely, linalool (27.26%) and methyl chavicol (19.77%), were the most important compounds [16]. In Algeria, Brada et al. identified the compositions of the essential oils of two varieties, *Ocimum basilicum* and *Ocimum basilicum gratissimum*. The first variety contained linalool (44.7%) as the primary component, while the second contained eugenol as the primary component [17]. Onofre et al. identified the following active ingredients in a sample from Romani: linalool, alphamuurolol, methyl chavicol, and eugenol [18]. The primary components of essential oil from Iran were estragole (18.80–50.32%) and 2, 6-octadienal (3.2–11.95%) [19]. Mota et al. reported that the main constituents of essential oil from the variety *Genovese Gigante* were eugenol (7.1–50.8%) and linalool (17–54.7%) [20], while Anwar et al. studied samples from different regions of Saudi Arabia and found estragole (60.88%) and linalool (25.33–363.09%) to be the principal components [21]. In addition, Mahmoud et al. identified linalool, methyl eugenol, methyl cinnamate, and estragole as the major compounds [22]. Phenylpropanoids were the predominant volatile fraction (72.5–77.5%) in the essential oil from Tunisia.
Figure 10: GC-MS chromatogram of essential oil of $A_t$.

Figure 11: Mass spectra of oleamide.
oil of *Ocimum basilicum* from India [23], while the major compound in the essential oil of basil from Vietnam was methyl chavicol [24]. The remarkable differences in the compositions of the essential oils of basil plants are attributable to the effects of geographical parameters, the nature of the soil and irrigation water used, and the season of growth.

4. Conclusion

The DOE approach was employed effectively to optimize the separation of complex mixtures by using temperature-programmed capillary GC. This study highlights the potential of DOE and, in particular, the importance of the Doehlert design strategy, which has proven to be a powerful tool that allows for the modelling of a large number of responses in a given experimental domain. In the temperature range that resulted in poor separation, the optimized conditions (slope = 1.06°C/min, final temperature = 121.41°C, and isotherm duration = 6.2 min) yielded satisfactory results, that is, it resulted in a large number of detected peaks and better resolution. Thus, this model should aid the development of improved methods for the analysis of essential oils. In addition, the acquired chromatograms constitute a database that can be used to build other models (within the experimental domain) for other pairs of compounds.

Based on the chemical and statistical analyses of the compositions of 24 essential oils of *O. basilicum*, three groups corresponding to the following compounds were detected: linalool, phenylpropanoid, and geranial. We also noticed that regardless of the variety of *O. basilicum* L and the region of the study investigated, linalool was always present, with its content varying from 52.1% (O5) to 5.5% (M3). This allowed us to use it as a marker of *O. basilicum* L. However, our attempt finds similarities in the compositions of the essential oils of six varieties of *O. basilicum* from four regions proved to be challenging, given the many factors that influence the composition.

Data Availability

All the data of our results are included in the text.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Supplementary Materials

This file contains two tables: Table 1 presents the results of the study of the dependent variables for the first factorial design. The variables and their boundary values were selected based on previous experience and typical values reported previously from analyses of *Ocimum basilicum* by using gas chromatography. Table 2 lists the Doehlert matrix for the three parameters (coded variables). This matrix consists of a set of 13 separate experiments. (Supplementary Materials)

References


