Hindawi
Journal of Chemistry
Volume 2022, Article ID 5880539, 14 pages
https://doi.org/10.1155/2022/5880539

Research Article

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

Nabila Slougui,[1,2] **Meriem Slama**,[2,3] **Yasser Boudaoud**,[2] **and Aymene Salah Bendrihem**[2]

[1]*Laboratoire de BioGéoChimie des Milieux Désertiques, Université Kasdi Merbah Ouargla, Route de Ghardaia, Ouargla 30000, Algeria*
[2]*Ecole Nationale Polytechnique de Constantine, Ville Universitaire Ali Mendjeli, BP 75A RP Ali Mendjeli, Constantine 25016, Algeria*
[3]*Laboratoire de Génie des Procédés pour le Développement Durable et Les Produits de Santé, École Nationale Polytechnique de Constantine, Constantine 25016, Algeria*

Correspondence should be addressed to Nabila Slougui; slougui.nabila@hotmail.com

Received 17 June 2022; Revised 16 September 2022; Accepted 5 October 2022; Published 13 October 2022

Academic Editor: Marcone A. L. de Oliveira

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 2^3 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. purpurescens* 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 (A_1).

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 spectroscopy, we used the DOE approach. The optimized model was then used to analyse 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_1) 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 $m \times 0.25$ mm id $\times 0.25\,\mu$m 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 $m \times 0.25$ 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, maintained at 280°C for 5 min, and finally increased to 330°C at a rate of 10°C/min for 2 min. The injector and detector (FID) temperatures were set to 330°C. The samples diluted in 1 μL of dichloromethane were injected in the split mode (30 : 1).

The GC-MS analysis was performed by using a GCMS-QP2010 system. The mass selective detector was equipped with an Rxi-5 ms capillary column (30 $m \times 0.25$ 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, then 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, maintained at 280°C for 5 min, and finally increased to 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 minimise the effect of potential errors. Each response was approximated by using a first-order polynomial regression function:

Table 1: Geographical data of four study regions.

	Ouargla (O)	Mostaganem (M)	Alger (A)	Constantine (C)
Latitude	31°97′	35°94′	36°58′	36°36′
Longitude	5°34′	0°09′	2°90′	6°60′
Altitude (m)	128	55	270	944

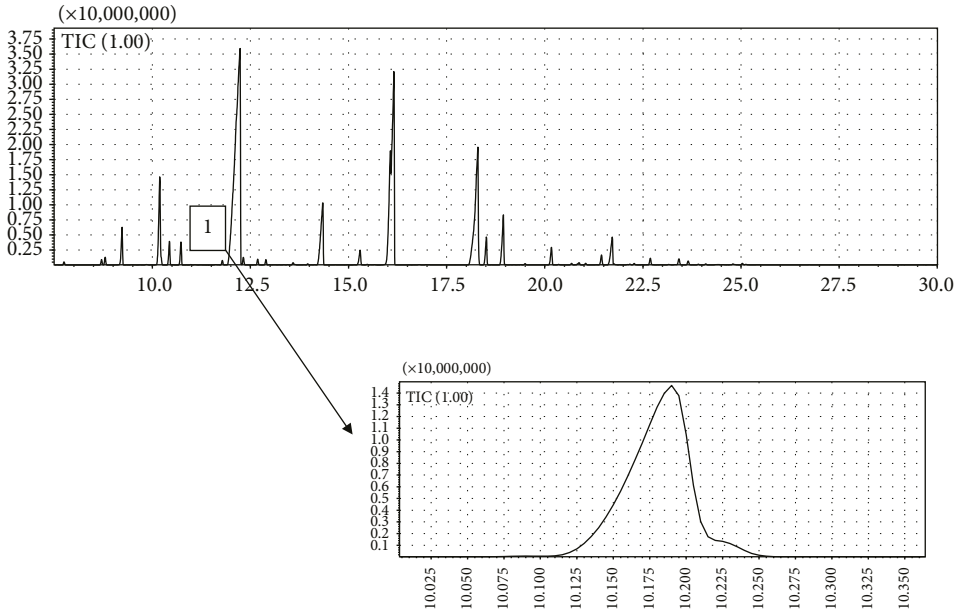


Figure 1: GC-MS chromatogram of basil essential oil sample before optimization (1-eucalyptol/D-limonene).

$$Y_i = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3, \quad (1)$$

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_I, 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_1X_2; A_{13} is the coefficient of interaction X_1X_3; and A_{23} is the coefficient of interaction X_2X_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.

Table 2: Compound pairs whose separation was optimized.

Compound pairs	Interference code	Response, Y_i
Sabinene/β-pinene	1	Y_1
Eucalyptol/D-limonene	2	Y_2
Geraniol/linalyl acetate	3	Y_3
Eugenol/α-terpinyl acetate	4	Y_4

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 3: Experimental design and method parameters used for the first two studies.

		First study	Second study
Experimental design		2^3 factorial design	Doehlert design
Factors/responses		3/2	3/4
Number of runs		11	18
Method parameters	Initial temperature	45°C	45°C
	Isotherm duration	5 min	5 and 10 min
	Slope	2 and 6°C/min	1 and 2°C/min
	Final temperature	180°C	80 and 180°C
	Isothermal step at FT	10 minutes	10 minutes
	Split ratio	$\frac{1}{40}$ and $\frac{1}{20}$	$\frac{1}{20}$
	Gas flow rate	0.6 and 1.3 mL/min	1.3 mL/min

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

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

$$Y_i = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{11}X_1^2 + A_{22}X_2^2 + A_{33}X_3^2 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3, \quad (2)$$

where Y_i is experimental resolution and is defined by

$$Y_i = \frac{2(t_2 - t_1)}{\delta_2 + \delta_1}, \quad (3)$$

where t_i is the retention time for eluted compounds 1 and 2 and δ_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

$$\mathrm{NP} = 113.88 + 15.63X_1 + 17.99X_2 + 16.38X_3 - 0.37X_1X_2 + 3.12X_1X_3 + 1.87X_2X_3. \quad (4)$$

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:

$$\mathrm{NP} = 116 + 17.75X_1 + 15.87X_2 + 18.5X_3. \quad (5)$$

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:

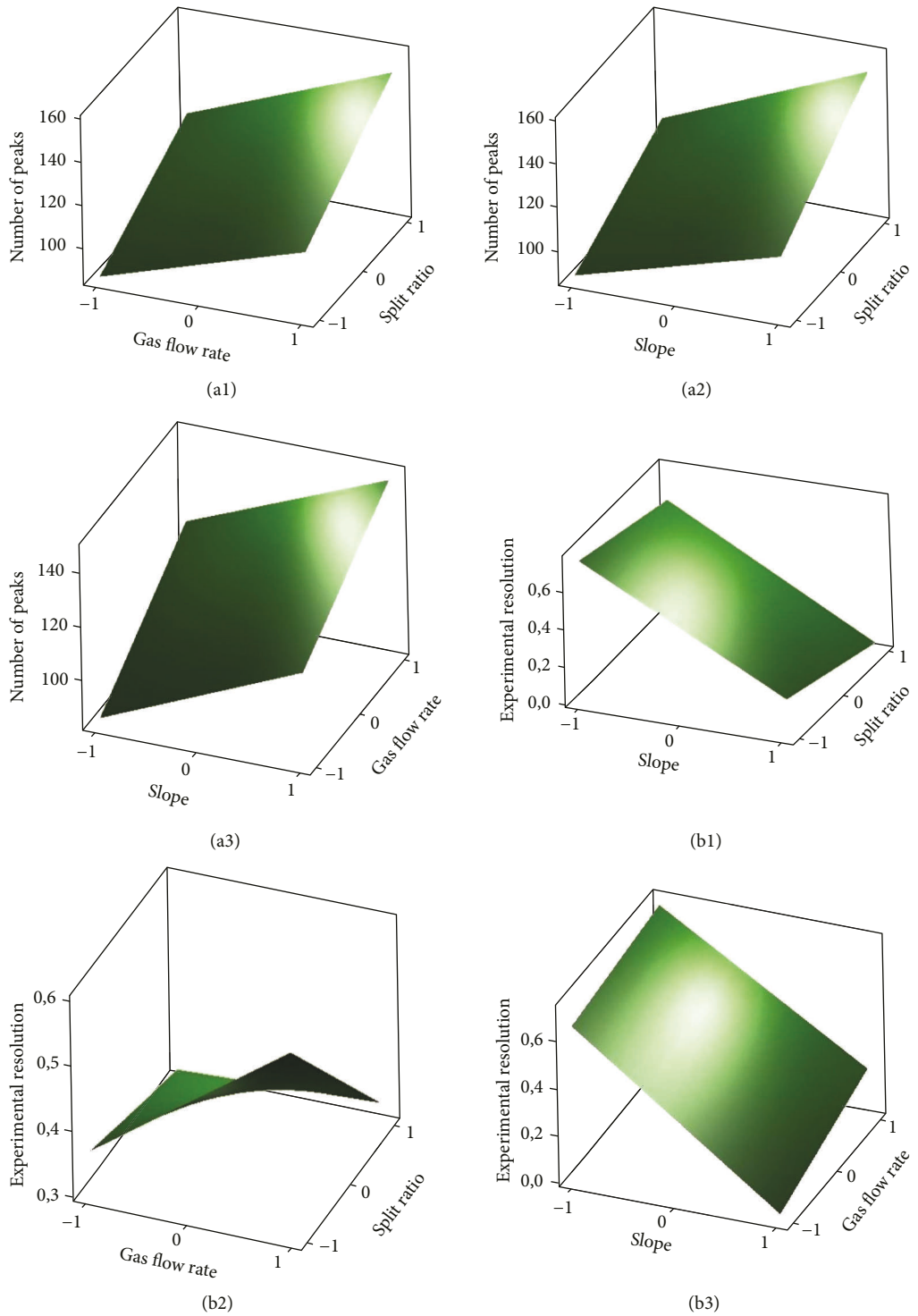


Figure 2: 3D surface plots of response showing variations in response: (a–c) number of peaks and (d–f) experimental resolution as functions of slope (°C/min), gas flow rate (mL/min), and split ratio.

$$\mathrm{ER} = 0.3710 - 0.2870X_1 + 0.0585X_2 - 0.0780X3 + 0.0255X_1X_2 - 0.0060X_1X_3 - 0.0505X_2X_3. \quad (6)$$

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:

Table 4: Effect estimates from first factorial design ($n = 8$).

		A coefficient	Std. err.	t value	p value
Number of peaks ($r^2 = 98.37\%$ Adj. $r^2 = 94.57\%$ $S = 5.994$)	Mean/Interact.	113.88	2.12	53.73	≤0.001
	X_1	15.63	2.12	7.37	0.005
	X_2	15.87	2.12	7.49	0.005
	X_3	16.38	2.12	7.73	0.871
	X_1 X_2	−0.37	2.12	−0.18	0.237
	X_1 X_2	3.12	2.12	1.47	0.441
	X_2 X_3	1.87	2.12	0.88	0.001
Experimental resolution ($r^2 = 98.18\%$ Adj. $r^2 = 93.93\%$ $S = 0.0693$)	Mean/Interact.	0.3710	0.0245	15.14	0.001
	X_1	−0.2870	0.0245	−11.71	0.097
	X_2	0.0585	0.0245	2.39	0.050
	X_3	−0.0780	0.0245	−3.18	0.375
	X_1 X_2	0.0255	0.0245	1.04	0.822
	X_1 X_2	−0.0060	0.0245	−0.24	0.131
	X_2 X_3	−0.0505	0.0245	−2.06	0.005

X_1: slope; X_2: gas flow rate; X_3: split ratio.

$$\mathrm{ER} = 0.3710 - 0.2870X_1 + 0.0585X_2 - 0.0780X_3 - 0.0505X_2X_3. \quad (7)$$

The variance values of $S = 0.0631$, $r^2 = 97.49\%$, and Adj. $r^2 = 94.97\%$ indicated good predictability.

Based on the above-described results, it was concluded that the selected factors, namely, X_1 (slope), X_2 (gas flow rate), and X_3 (split ratio), are important for the regression model. Furthermore, based on the above-described response surface plots, the optimal regions were selected to be −1, +1, and +1 for the slope (2°C/min), gas flow rate (1.3 mL/min), and split ratio (1/20), respectively. The desirability, D, was 0.6817.

The Pareto charts of the standardized effect estimates for the chromatographic responses are given in Figure 3, while Figure 4 shows the four parts of the GC-MS chromatogram of a basil essential oil sample obtained under optimal conditions.

3.1.2. Second Study. The optimal conditions obtained from the first study were used for the second study. Table 5 shows the experimental design derived from the Doehlert matrix and the experimental values listed in Table 3. The experimental responses for each interference are also listed in Table 5.

The polynomial models for the four responses were validated by ANOVA. In other words, the experimental and predicted values were in good agreement. Contour plots are drawn for each interference (Figure 5) for the entire experimental domain to obtain richer data.

Finally, the best separation of the four pairs of compounds could be achieved. Calculations were performed by using Minitab 18, and the optimal conditions (Table 6) were determined, with the global degree of satisfaction for the four responses being equal to 98%.

The validity of Equation (8) for describing Y_1 as a function of the slope, final temperature, and isotherm duration was confirmed statistically. It was also determined that the initial model could be reduced to a final four-parameter model of the following form:

$$Y_1 = 0.644 - 0.09021\mathrm{S} - 0.01633\mathrm{ID} - 0.0319\mathrm{FT}^2. \quad (8)$$

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:

$$Y_2 = 0.89671 - 0.08732\mathrm{S} - 0.02654\mathrm{ID} - 0.0368\mathrm{S}^2 - 0.0284\mathrm{FT}^2 + 0.0490\mathrm{FT} * \mathrm{ID}. \quad (9)$$

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.

$$Y_3 = 0.85579 - 0.11403\mathrm{S} - 0.02316\mathrm{FT}^2 - 0.02289\mathrm{ID}^2. \quad (10)$$

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:

$$Y_4 = 0.25595 - 0.09454\mathrm{S} - 0.02714\mathrm{FT}^2 + 0.03786\mathrm{S}^2. \quad (11)$$

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.

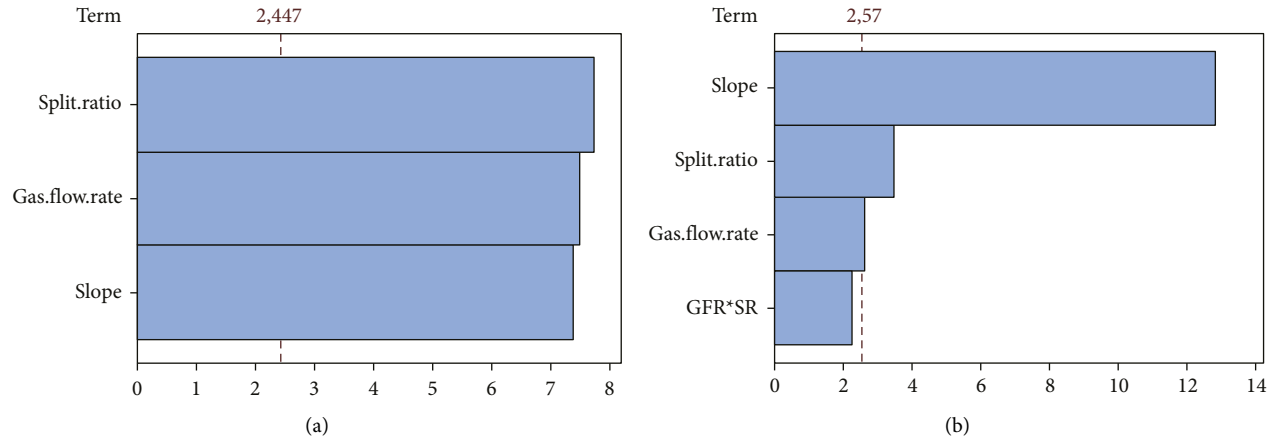


Figure 3: Pareto charts of standardized effect estimates of chromatographic responses. (a) The number of peaks. (b) Experimental resolution.

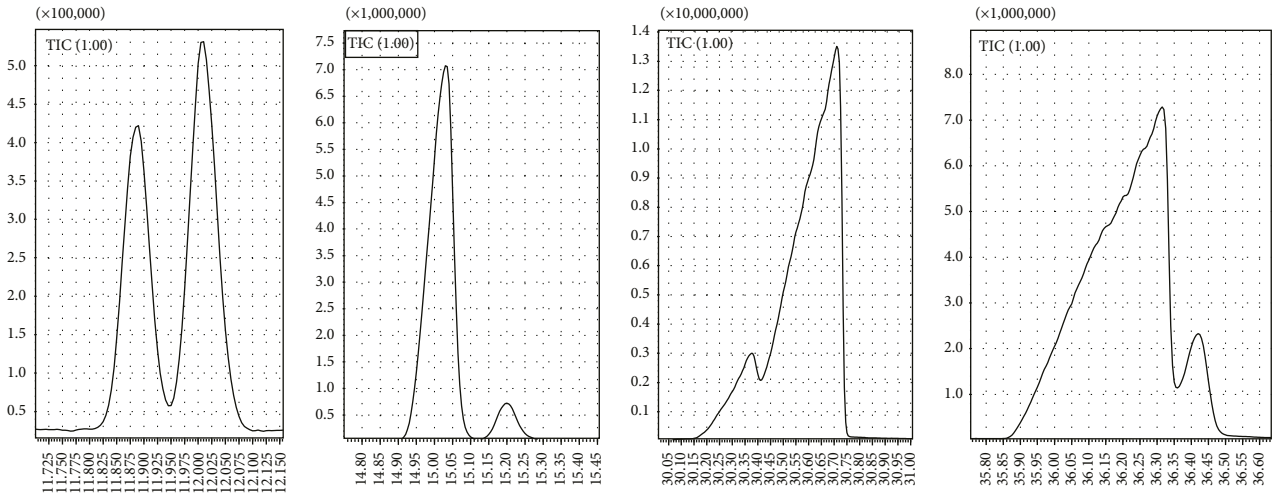


Figure 4: Parts of GC-MS chromatogram for basil essential oil sample obtained under optimal conditions. Slope = 2°C/min, gas flow rate = 1.3 mL/min, and split ratio = 1/20. From left to right, (1) sabinene/β-pinene, (2) eucalyptol/D-limonene), (3) geraniol/linalyl acetate), and (4) eugenol/α-terpinyl acetate).

Table 5: Experimental design and experimental resolutions (Y_i).

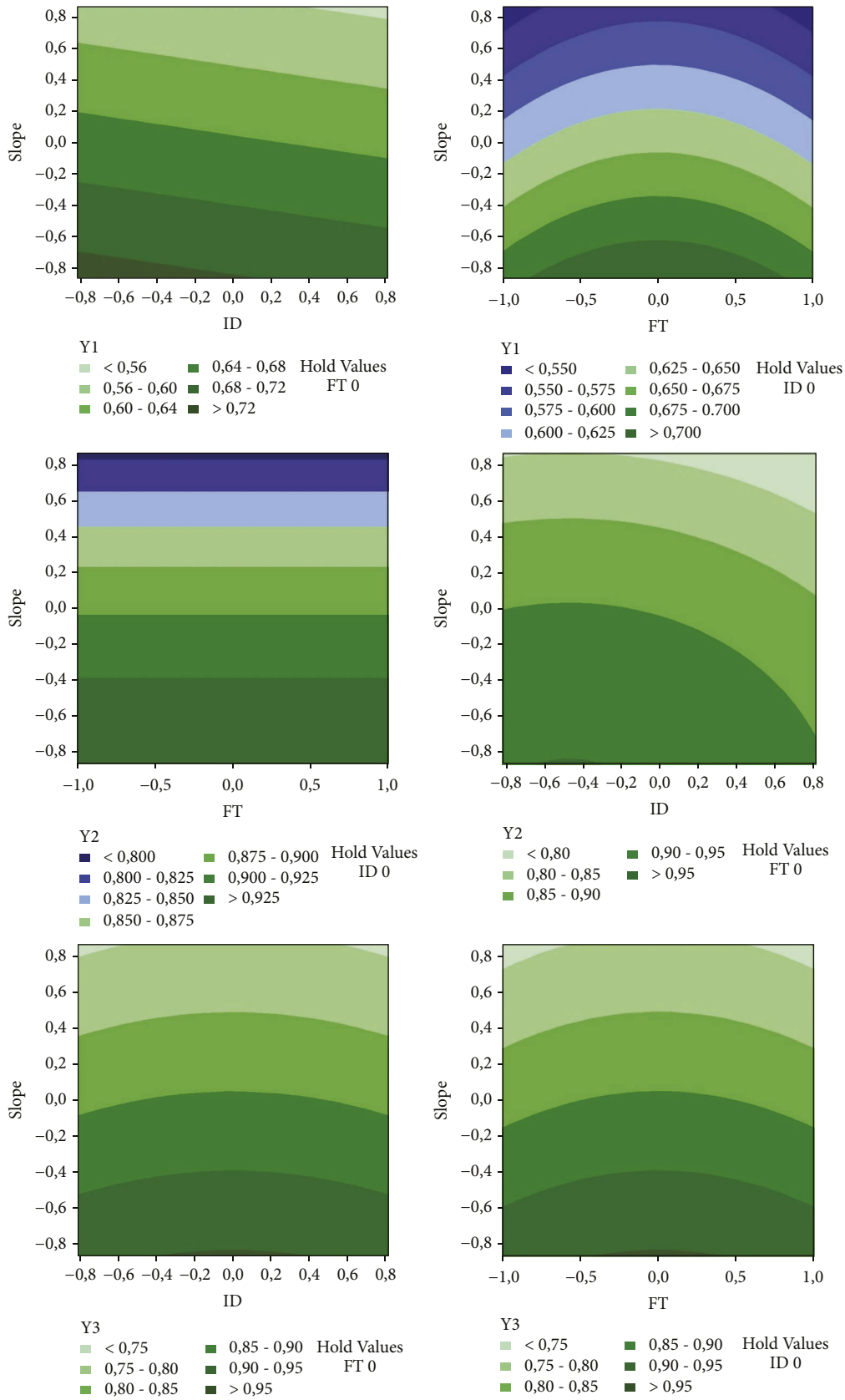
Final temperature	Slope	Isotherm duration	Y_1	Y_2	Y_3	Y_4
130	1.50	7.50	0.63	0.90	0.86	0.26
180	1.50	7.50	0.60	0.88	0.84	0.24
80	1.50	7.50	0.62	0.89	0.83	0.22
155	1.93	7.50	0.55	0.79	0.74	0.19
105	1.06	7.50	0.73	0.96	0.94	0.37
155	1.06	7.50	0.72	0.94	0.95	0.35
105	1.93	7.50	0.57	0.78	0.75	0.20
155	1.64	9.54	0.60	0.86	0.81	0.22
105	1.35	5.46	0.66	0.92	0.87	0.28
155	1.35	5.46	0.68	0.90	0.85	0.26
130	1.78	5.46	0.63	0.86	0.77	0.21
105	1.64	9.54	0.60	0.80	0.81	0.24
130	1.21	9.54	0.69	0.89	0.91	0.33
130	1.50	7.50	0.65	0.89	0.88	0.24
130	1.50	7.50	0.63	0.90	0.85	0.25
130	1.50	7.50	0.65	0.90	0.84	0.25
130	1.50	7.50	0.63	0.92	0.87	0.28
130	1.50	7.50	0.63	0.90	0.85	0.26

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 (O_1, M_1, M_2, O_3, M_4, C_5, O_5, O_4, C_4, M_5 and M_4, which are green); phenylpropanoid group: this group (eugenol/methyl eugenol/methyl chavicol/methyl cinnamate)



(a)

Figure 5: Continued.

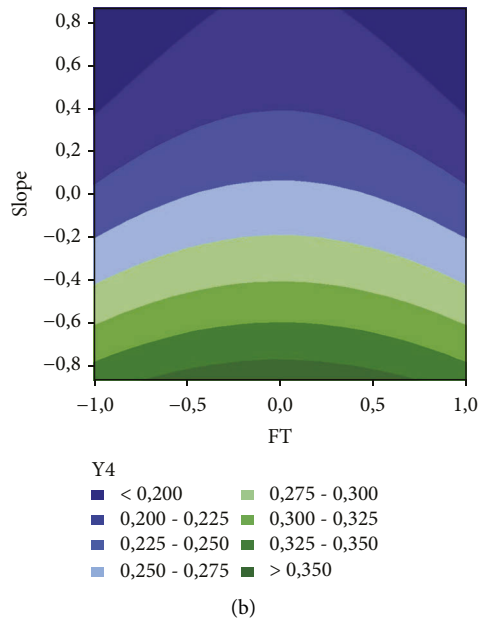


Figure 5: Contour plots for four interferences for the entire experimental domain.

Table 6: Predicted optimal conditions for analysis of essential oils from basil.

Parameter	Value
Slope (°C/min)	1.06
Final temperature (°C)	121.41
Isotherm duration (min)	6.2

Table 7: Predicted and experimental resolutions for 12 interferences listed in Table 3 under optimal conditions: ID = 6.2 min, $S = 1.06$ °C/min, and FT = 121.41°C.

Interference to be resolved	Resolution predicted	Experimental
Sabinene/β-pinene	0.73	0.65
Eucalyptol/D-limonene	0.95	0.96
Geraniol/linalyl acetate	0.95	0.96
Eugenol/α-terpinyl acetate	0.36	0.32

contains nine essential oils (C_3, M_3, A_2, A_3, C_2, A_1, O_2, A_5, and C_1, which are red); and geranial group: this group contains four essential oils, namely, those of lemon basil (A_6, C_6, M_6, and O_6, 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 (A_1). Figure 10 shows the chromatogram of the essential oil of A_1, 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

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

Model	Equation (8)	Equation (9)	Equation (10)	Equation (11)
Variance	0.0133725 (17)	0.0148353 (17)	0.0115758 (17)	0.0122119 (17)
Pure error variance	0.000533 (5)	0.000483 (5)	0.001083 (5)	0.000933 (5)
Lack-of-fit variance	0.001970 (9)	0.002158 (7)	0.00793 (9)	0.001155 (9)
$F_{calculated}$	65.72	34.60	133.26	87.98
$F_{estimated}$	3.34	3.11	3.34	3.34
r^2	93.37%	93.51%	96.62%	94.96%

Degrees of freedom are listed within parentheses.

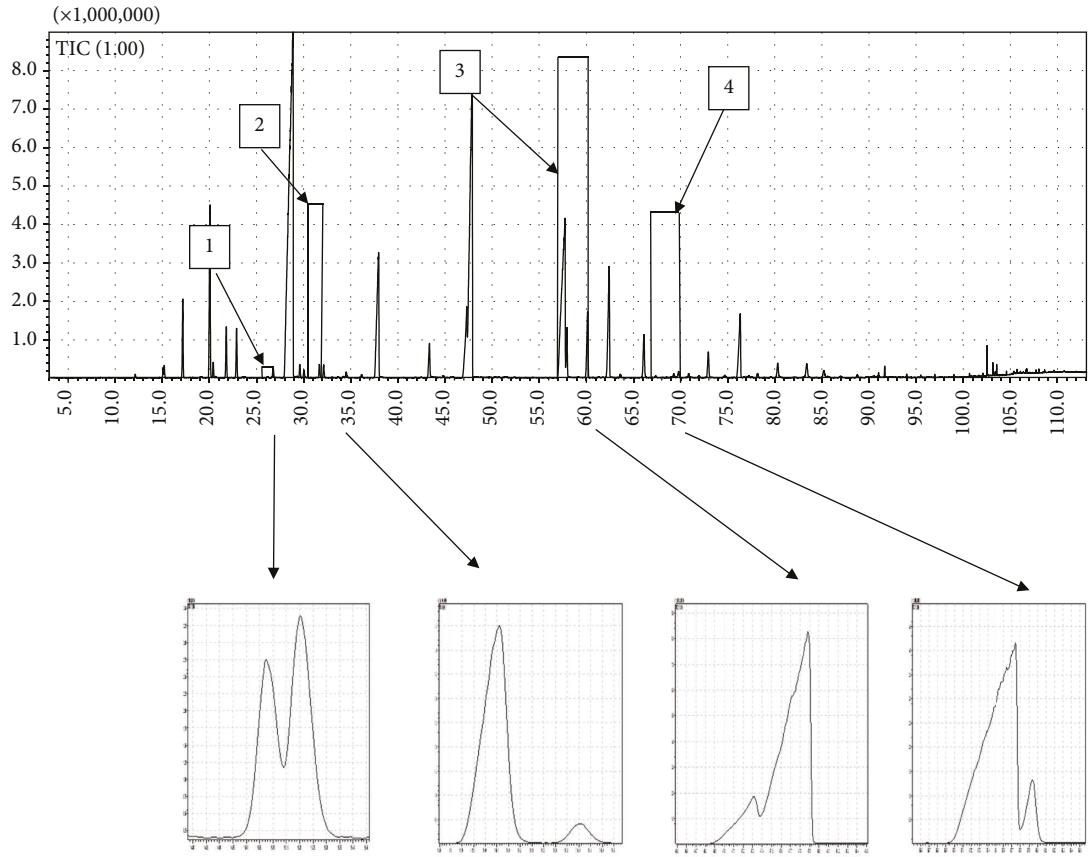


Figure 6: GC-MS chromatogram of basil essential oil sample obtained under optimal conditions. Slope = 1.06°C/min, final temperature = 121.41°C, and isotherm duration = 6.2 min. From left to right, (1) sabinene/β-pinene), (2) eucalyptol/D-limonene), (3) geraniol/linalyl acetate), and (4) eugenol/α-terpinyl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

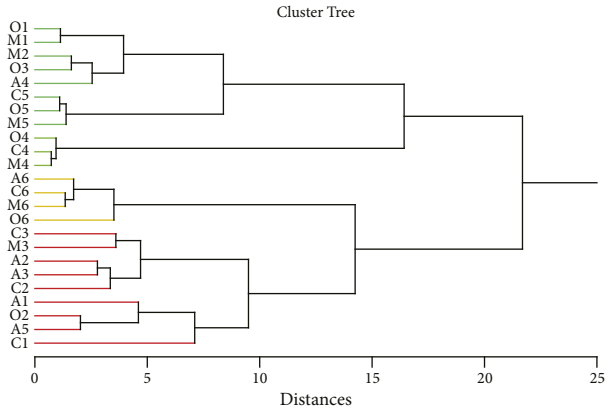


Figure 7: Cluster tree of 24 essential oils studied.

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

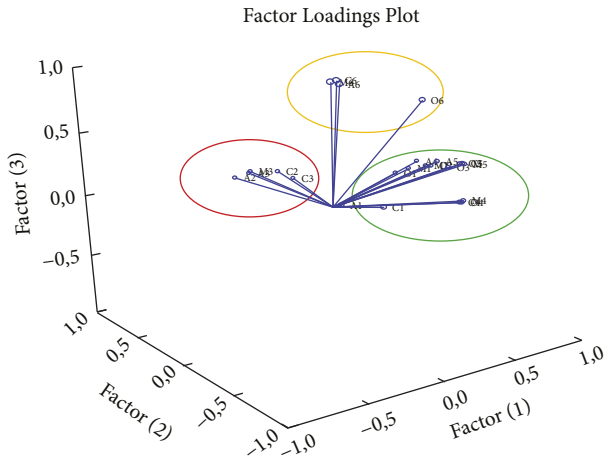


Figure 8: 3D PCA results for all essential oils studied.

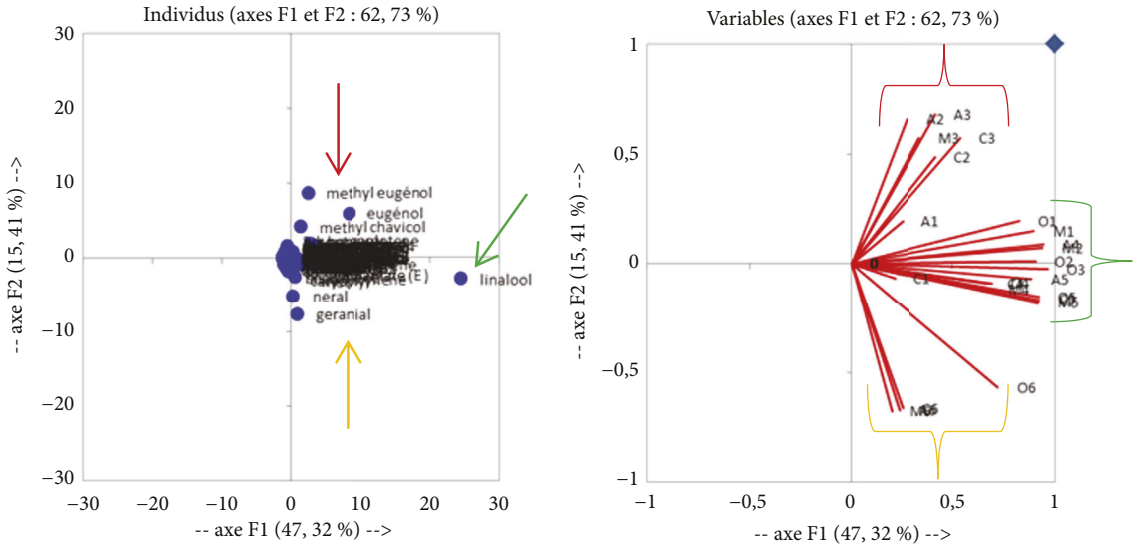


Figure 9: PCA results for individual components and various variables for all essential oils studied.

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]. Onofrei et al. identified the following active ingredients in a sample from Romani: linalool, alpha-muurolol, 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

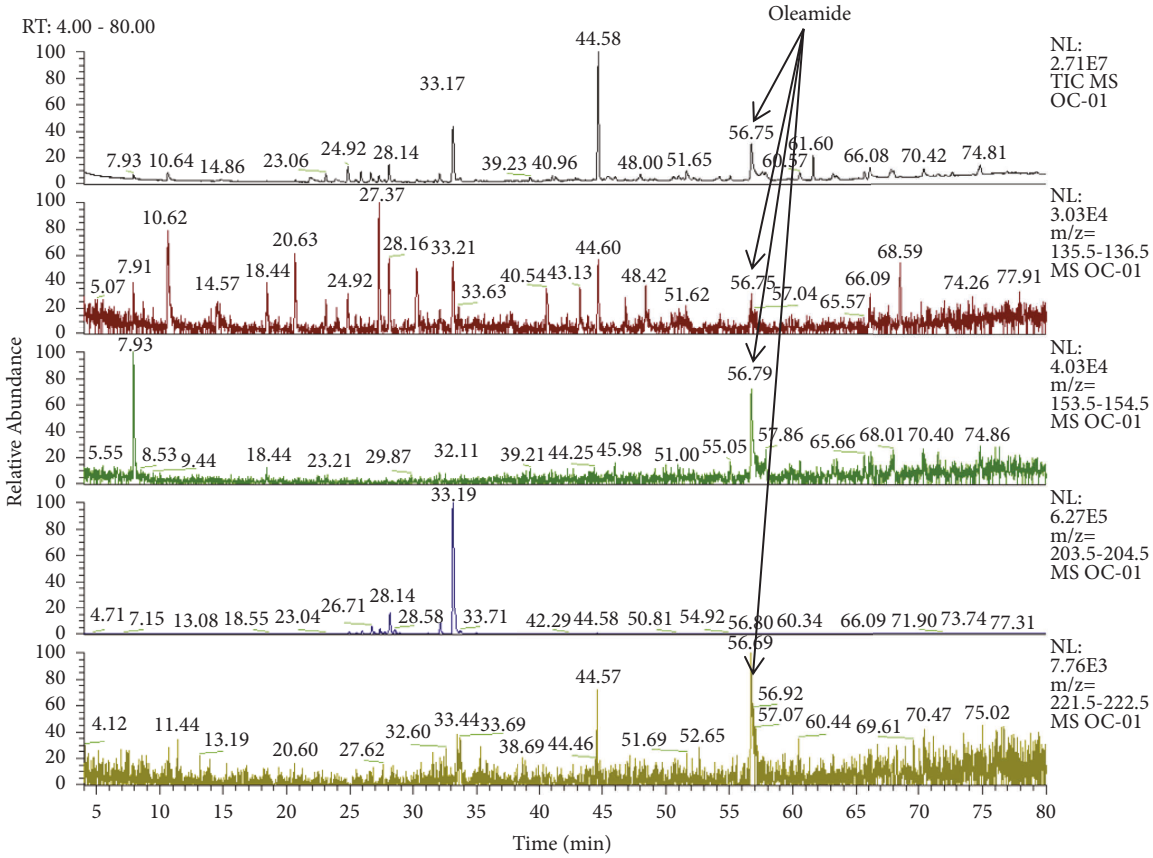


Figure 10: GC-MS chromatogram of essential oil of A_1.

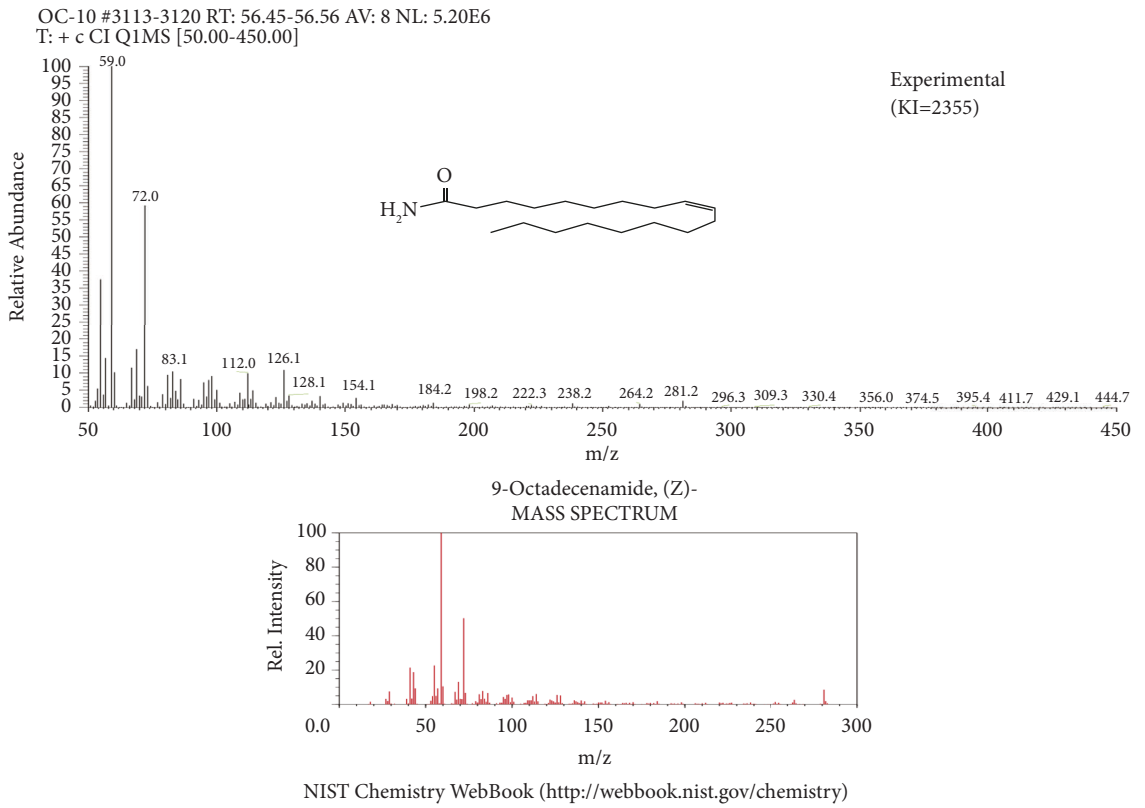


Figure 11: Mass spectra of oleamide.

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% (O_5) to 5.5% (M_3). 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

[1] A. S. Ventura, G. T. Jerônimo, R. A. C. Corrêa Filho et al., "*Ocimum basilicum* essential oil as an anesthetic for tambaqui *Colossoma macropomum*: hematological, biochemical, non-specific immune parameters and energy metabolism," *Aquaculture*, vol. 533, Article ID 736124, 2021.

[2] M. Cai, Y. Wang, R. Wang et al., "Antibacterial and anti-biofilm activities of chitosan nanoparticles loaded with *Ocimum basilicum* L. essential oil," *International Journal of Biological Macromolecules*, vol. 202, pp. 122–129, 2022.

[3] K. Hubai, O. Székely, G. Teke, and N. Kováts, "Is essential oil production influenced by air pollution in *Ocimum basilicum* L," *Biochemical Systematics and Ecology*, vol. 96, Article ID 104248, 2021.

[4] H. Kahveci, N. Bilginer, E. Diraz-Yildirim et al., "Priming with salicylic acid, β-carotene and tryptophan modulates growth, phenolics and essential oil components of *Ocimum basilicum* L. grown under salinity," *Scientia Horticulturae*, vol. 281, Article ID 109964, 2021.

[5] R. Gupta, A. Singh, R. Kanaujia, S. Kushwaha, and R. Pandey, "Trichoderma harzianum ThU and its metabolites underscore alteration in essential oils of *Ocimum basilicum* and *Ocimum sanctum*," *Proceedings of the National Academy of Sciences, India—Section B: Biological Sciences*, vol. 88, no. 1, pp. 219–227, 2018.

[6] V. Gradinariu, O. Cioanca, L. Hritcu, A. Trifan, E. Gille, and M. Hancianu, "Comparative efficacy of *Ocimum sanctum* L. and *Ocimum basilicum* L. essential oils against amyloid beta (1–42)-induced anxiety and depression in laboratory rats," *Phytochemistry Reviews*, vol. 14, no. 4, pp. 567–575, 2015.

[7] C. Osei Akoto, A. Acheampong, Y. D. Boakye, A. A. Naazo, and D. H. Adomah, "Anti-Inflammatory, antioxidant, and anthelmintic activities of *Ocimum basilicum* (sweet basil) fruits," *Journal of Chemistry*, vol. 2020, Article ID 2153534, 9 pages, 2020.

[8] N. A. Alamrani, H. A. Al-Aoh, M. M. H. Aljohani et al., "Wastewater purification from permanganate ions by sorption on the *Ocimum basilicum* leaves powder modified by zinc chloride," *Journal of Chemistry*, vol. 2021, Article ID 5561829, 10 pages, 2021.

[9] N. Khalediyan, W. Weisany, and P. M. Schenk, "Arbuscular mycorrhizae and rhizobacteria improve growth, nutritional status and essential oil production in *Ocimum basilicum* and Satureja hortensis," *Industrial Crops and Products*, vol. 160, Article ID 113163, 2021.

[10] Y. Gökçe, H. Kanmaz, B. Er, K. Sahin, and A. A. Hayaloglu, "Influence of purple basil (*Ocimum basilicum* L.) extract and essential oil on hyperlipidemia and oxidative stress in rats fed high-cholesterol diet," *Food Bioscience*, vol. 43, 2021.

[11] E. M. de Souza, R. C. de Souza, J. F. Melo, M. M. da Costa, A. M. de Souza, and C. E. Copatti, "Copattia. Evaluation of the effects of *Ocimum basilicum* essential oil in Nile tilapia diet: growth, biochemical, intestinal enzymes, haematology, lysozyme and antimicrobial challenges," *Aquaculture*, vol. 504, no. 15, pp. 7–12, 2019.

[12] R. ., P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Pub Corp, Carol Stream, IL, USA, 4th edition, 2007.

[13] S. L. C. Ferreira, W. N. L. Dos Santos, C. M. Quintella, B. B. Neto, and J. M. Bosque-Sendra, "Doehlert matrix: a chemometric tool for analytical chemistry—Review," *Talanta*, vol. 63, no. 4, pp. 1061–1067, 2004.

[14] B. d Barros Neto, I. S. Scarminio, and R. E. Bruns, "25 anos de quimiometria no Brasil," *Química Nova*, vol. 29, no. 6, pp. 1401–1406, 2006.

[15] W. M. F. da Silva, D. H. Kringel, E. J. D. de Souza, E. da Rosa Zavareze, and A. R. G. Dias, "Basil essential oil: methods of extraction, chemical composition, biological activities, and food applications," *Food and Bioprocess Technology*, vol. 15, no. 1, pp. 1–27, 2022.

[16] S. Shatar, S. Altantsetseg, I. Sarnai, D. Zoljargal, T. D. Thang, and N. X. Dung, "Chemical composition of the essential oil of *Ocimum Basilicum* cultivated in Mongolian Desert-Gobi," *Chemistry of Natural Compounds*, vol. 43, no. 6, pp. 726-727, 2007.

[17] M. Brada, L. H. Khelifa, D. Achour, J. P. Wathelet, and G. Lognay, "Essential oil composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Algeria," *Journal of Essential Oil Bearing Plants*, vol. 14, no. 6, pp. 810–814, 2011.

[18] V. Onofrei, A. Benchennouf, M. Jancheva et al., "Ecological foliar fertilization effects on essential oil composition of sweet basil (*Ocimum basilicum* L.) cultivated in a field system," *Scientia Horticulturae*, vol. 239, pp. 104–113, 2018.

[19] B. Fattahi, K. Arzani, M. K. Souri, and M. Barzegar, "Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (*Ocimum basilicum* L.)," *Industrial Crops and Products*, vol. 138, Article ID 111584,, 2019.

[20] I. Mota, J. Sánchez-Sánchez, L. G. Pedro, and M. J. Sousa, "Composition variation of the essential oil from *Ocimum basilicum* L. cv. *Genovese Gigante* in response to Glomus intraradices and mild water stress at different stages of growth," *Biochemical Systematics and Ecology*, vol. 90, Article ID 104021, 2020.

[21] F. Anwar, K. M. Alkharfy, T. Mehmood, M. A. Bakht, and R. Najeeb-ur, "Variation in chemical composition and effective antibacterial potential of *Ocimum basilicum* L. essential oil harvested from different regions of Saudi Arabia," *Pharmaceutical Chemistry Journal*, vol. 55, no. 2, pp. 187–193, 2021.

[22] E. Mahmoud, M. Starowicz, E. Ciska, J. Topolska, and A. Farouk, "Determination of volatiles, antioxidant activity, and polyphenol content in the postharvest waste of *Ocimum basilicum* L," *Food Chemistry*, vol. 375, Article ID 131692, 2022.

[23] S. Srivastava, R. K. Lal, K. Yadav et al., "Chemical composition of phenylpropanoid rich chemotypes of *Ocimum basilicum* L. and their antimicrobial activities," *Industrial Crops and Products*, vol. 183, Article ID 114978, 2022.

[24] J. H. Miyoshi, J. C. Castro, V. C. Fenelon et al., "Essential oil characterization of *Ocimum basilicum* and *Syzygium aromaticum* free and complexed with β-cyclodextrin. Determination of its antioxidant, antimicrobial, and antitumoral activities," *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, vol. 102, no. 1-2, pp. 117–132, 2022.