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(Research Article)

Effect of temperature programming on separation and quantitative analysis of organics using gas chromatography-flame ionization detector

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Abstract

The temperature of the column constitutes a crucial parameter in gas chromatography experiments and necessitates precise control for accurate investigation. Adjustment of temperature is performed to induce alterations in the equilibrium, thereby affecting the retention time throughout the analysis. The current investigation successfully examined the impact of temperature programming on separations of natural products such as hydrocarbons, as well as their analysis in a commercial cleaning solvent (Wessex Triple X) using gas chromatography-flame ionization detector. The findings indicate that the effectiveness of separation is significantly influenced by the column temperature, with the gradient program (40°C to 100°C at 20°C/min) proving to be more efficient than isothermal programs (40°C and 50°C). Furthermore, qualitative and quantitative analysis of a commercial cleaning solvent revealed the presence of cyclohexane and p-xylene, with concentrations of 193±2 ppm and 359±5 ppm, respectively. Enhanced values for N_{plates}, the height equivalent of theoretical plates, and resolution were achieved with the implementation of a gradient temperature program. Thus, leading to improved separation.

Keywords: Gas chromatography; Organics; Column temperature; Gradient temperature; Isothermal

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1. Introduction

Gas chromatography (GC) is a highly reliable and adaptable method that can effectively separate intricate mixtures by exploiting differences in boiling points, vapor pressures, and polarities. Its widespread application across various industrial fields like petroleum, pharmaceuticals, and environmental sciences stems from its proficiency in detecting residual impurities and quantifying different hydrocarbon components in oils and fuels [1], [2]. Additionally, it is employed in food analysis to profile fatty acids and identify resin acids in water and air pollutants. The separation process in GC takes place within a column coated with a stationary phase, typically polydimethylsiloxane (PDMS). The volatile compounds being analyzed interact with this stationary phase on the column walls, causing specific compounds to elute at distinct time points due to their unique chemical properties, thus establishing their retention time [3]. Qualitative analysis of GC is achieved by comparing these retention times. The carrier gas (helium or nitrogen) transports the vaporized components to the detector. Many detectors, such as the electron capture detector (ECD), nitrogen and phosphorus detector (NPD), and mass spectrometry detector (MSD), are used as detectors [2]. While various detectors can be used with GC, the flame ionization detector (FID) is predominantly favored for analyzing organic molecules and hydrocarbons due to its exceptional performance. Operating with a hydrogen-air flame, the FID combusts the sample to oxidize organic molecules, producing ions (CHO^+) that are then collected to generate an electrical signal for measurement. Notably, GC-FID exhibits high sensitivity with an impressively low detection limit in

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the picogram range, a wide linear working range, and the ability to analyze almost all organic compounds except CO_2 and CO [4].

The temperature of the column is a critical factor in GC experiments and must be precisely controlled to ensure accurate analysis. Regulating the temperature in GC allows for adjustments that influence the equilibrium and, consequently, the retention time of compounds [5]. The fundamental principle of separation in GC relies on compound volatility, where those with lower volatility may not vaporize, leading to delayed elution or extended retention times at lower temperatures [6], [7]. Isothermal conditions entail maintaining a constant temperature throughout the separation process, while gradient conditions involve a temperature change during separation. This temperature gradient aids in the effective separation of compounds with different volatilities. Therefore, optimizing conditions is crucial [1]. Despite extensive research on factors like isothermal temperature, carrier gas flow rate, injection split ratio, and carrier gas composition in various applications, there is still considerable potential for investigating the effects of temperature variations and injection holding times. Thus, this study utilized GC-FID to explore the impact of temperature programming on compound separation. Additionally, a quantitative analysis of specific organic compounds (cyclohexane, toluene, p-xylene, and o-xylene) in a commercial cleaning solvent was conducted.

2. Materials and methods

2.1. Material

Pentane, cyclohexane, toluene, p-xylene, and o-xylene with purities of 99 %, 99.5 %, 99 %, 99 % and 99 % respectively, were purchased from Merck (Darmstadt, Germany). A commercial cleaning solvent (Wessex Triple X) was purchased from Tesco 24, Aberdeen, UK. Solvents used in this study were brought to room temperature before use. The standard solutions of organic solvents in respective concentrations were prepared by diluting the standard stock with deionized water.

2.2. Instrumentation

All experiments were performed on an Agilent Technologies 7820A gas chromatograph equipped with a flame ionization detector (GC-FID) and an integrated autoinjector. The experimental conditions are shown in Table 1. A 5 μL syringe was used in injecting the samples. The syringe was carefully pierced into the sample vials, in which 1 μL was carefully taken, and it was ensured no air bubbles were in the syringe. The sample was then gently injected at the injection port, and the start button on the instrument was pressed as the sample vaporized immediately. A chromatogram was generated displaying the elution times of different compounds and peak area according to their respective boiling points. The sample procedure was repeated throughout the experiment. Pentane was used as the blank and injected first to check whether impurities are present, which can affect results and retention times of the identified compounds.

Table 1 Experimental conditions for effect of temperature on separation

Operating conditions	Injection type/concentration	Operating Temperature	Expected compounds present
Isothermal 50	1000 ppm mixed standard	Constant temperature at 50 °C	Cyclohexane, toluene, p-xylene, and o-xylene.
Isothermal 40		Constant temperature at 40 °C	
Gradient 1		40 °C to 100 °C with a constant gradient of 20 °C/min	
Petrol gradient	Cleaning solvent	40 °C to 200 °C with temperature gradient of 20 °C/min	p-xylene and cyclohexane

The height equivalent of theoretical plates (HETP), a crucial parameter determining separation efficiency, and the number of theoretical plates (Nplates), also used to compare column efficiency following temperature change, were calculated using Equations (I) and (II) [4], [8]. The results obtained are depicted in Table 2.

$$N_{plates} = 16*(\frac{t_r}{W})^2 \qquad \dots\dots\dots\dots\dots\dots \quad (I)$$

Here, t_r represents retention time, and W is the peak width.

$$HETP = \frac{L_{column}}{N_{plates}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (II)$$

where L_{column} represents the column's length

Additionally, the resolution (R) between adjacent peaks was determined using Equation (III).

$$R = \frac{2(tr_2 - tr_1)}{w_1 + w_2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (III)$$

Where tr_2 is the retention time of the second eluting compound, tr_1 is the retention time for the first eluting compound, and w_1 and w_2 are the peak widths of compounds 1 and 2.

3. Results and discussion

3.1. Effect of temperature programming on separation efficiency

Table 2 demonstrates the impact of temperature programming on the separation of compounds. A compound with a low boiling point is anticipated to volatilize easily and elute before those with higher boiling points, indicating less volatility and later elution. Under isothermal conditions, where the column temperature remains constant, the most volatile compound is expected to elute first, followed by the least volatile [9]. Decreasing the temperature from 50°C to 40°C resulted in increased retention times for individual compounds, as more time is needed to reach their boiling points, as shown in Figures 1, 2, and 3. A higher N_{plates} value indicates a greater likelihood of two compounds being separated [4]. From the results obtained, it can be noted that N_{plates} increased for each compound when the temperature decreased from 50°C to 40°C, indicating an enhanced column efficiency. Consequently, altering the temperature also affects HETP, as illustrated in Table 2. HETP values decreased for each compound when the temperature was reduced from 50°C to 40°C, with lower HETP values indicating higher separation efficiency. The chromatogram peaks are well-separated, and no peak overlap is expected based on the compounds' boiling points, although some improvement was observed when the temperature was lowered. When comparing isothermal temperature programs (40°C and 50°C) with a gradient program (40°C to 100°C at 20°C/min), the gradient program allowed for faster separation due to ramping.

It is apparent that a reduction in peak width occurs with the utilization of a gradient temperature program, suggesting the generation of narrower peaks as opposed to broader ones. In the absence of a gradient program and with a constant column temperature, the initial peaks to emerge may be well distinguished; however, subsequent components may linger longer on the stationary phase, leading to peak broadening and extended run times. Conversely, employing a gradient temperature allows for swifter elution of tardy components, thus preventing peak broadening [2]. Consequently, the most effective approach for achieving optimal separation is through the implementation of a gradient program.

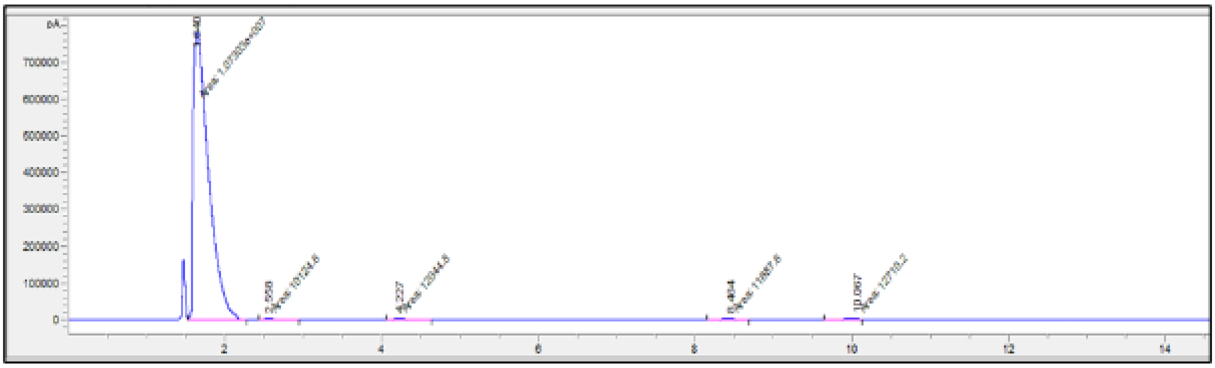

Figure 1 Chromatogram for isothermal 40

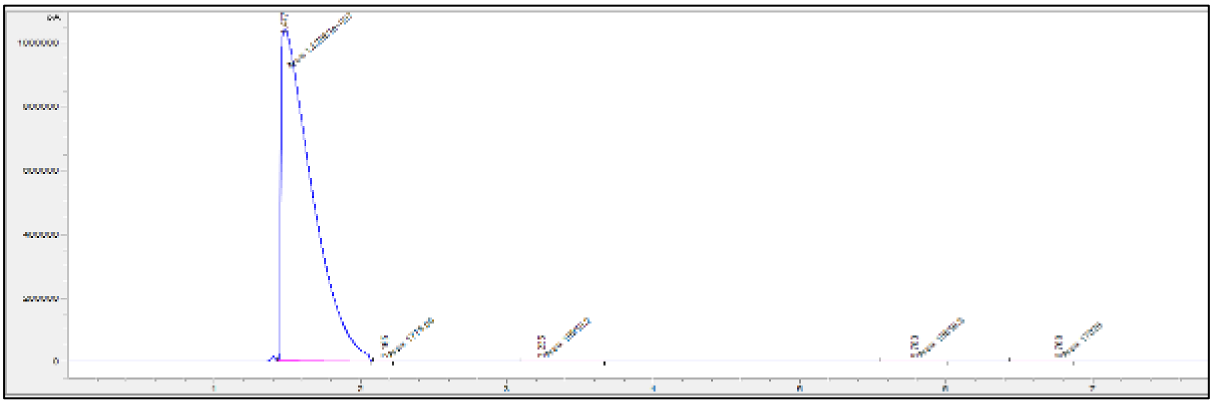

Figure 2 Chromatogram for isothermal 50

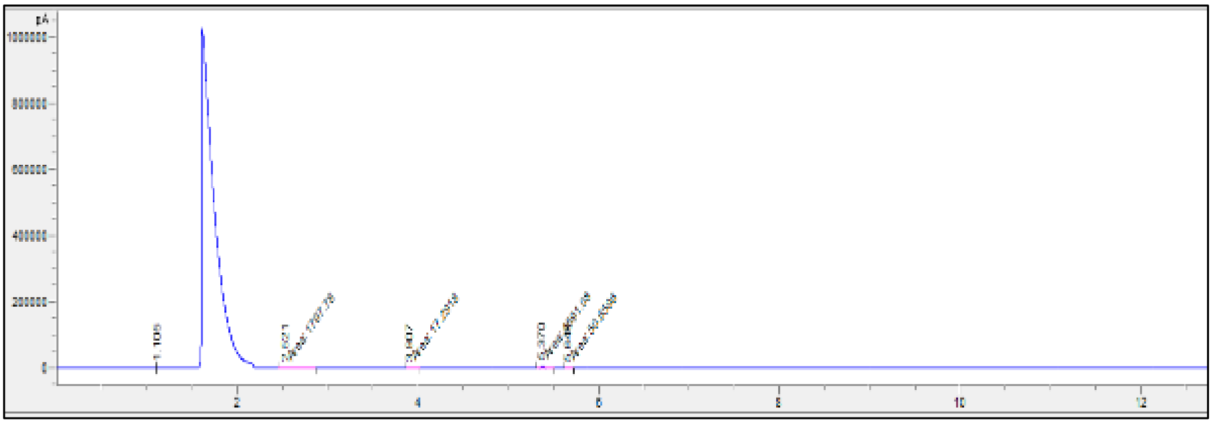

Figure 3 Chromatogram for gradient program

Table 2 Results from different temperature programming system

Results of 50°C Isothermal run							
Peak No	Compound	t_R	t_W	Peak area	N_{plates}	HETP	R
1	Pentane	1.477	0.245	15258924	583	0.051	-
2	Cyclohexane	2.165	0.093	1779	8746	0.003	4.080
3	Toluene	3.235	0.238	16958	2956	0.010	6.473
4	P-xylene	5.783	0.227	15859	10347	0.003	10.94
5	O-xylene	6.768	0.199	17026	18414	0.002	4.614
Results of 40°C isothermal run							
Peak No	Compound	t_R	t_W	Peak area	N_{plates}	HETP	R
1	Pentane	1.646	0.228	10730274	836	0.036	-
2	Cyclohexane	2.558	0.178	10124	3293	0.009	4.492
3	Toluene	4.227	0.193	12044	7643	0.004	8.981
4	P-xylene	8.464	0.217	11688	24386	0.001	20.65
5	O-xylene	10.07	0.255	12710	24936	0.001	6.795

Results of gradient run (40 °C – 100 °C) at 20°C/min							
Peak No	Compound	t_R	t_W	Peak area	N_{plates}	HETP	R
1	Pentane	1.646	0.226	10346383	843	0.036	-
2	Cyclohexane	2.563	0.146	6984	4917	0.006	4.916
3	Toluene	3.945	0.113	10149	19432	0.002	10.65
4	P-xylene	5.400	0.062	11399	122160	0.0002	16.63
5	O-xylene	5.705	0.046	12849	246102	0.0001	5.658

3.2. Quantification of aromatic compounds in commercial cleaning solvent

Table 3 Qualification data for cleaning solvent analysis

Single compound standard	**t_R/min single standard**	**t_R/min mixed standard**	**t_R difference**	**Acceptable**
P-xylene	5.322	5.375	0.05	yes
Toluene	3.895	3.914	0.02	yes

Prior to the analysis of the cleaning solvent to identify its constituent compounds, a qualifying examination was conducted. Two distinct single standard solutions, each with a concentration of 1000 ppm, were subjected to analysis using the refined gradient temperature method. This comparison aimed to assess the retention times in relation to those of the 1000 ppm mixed standards. The findings, depicted in Table 3, indicated that the retention time discrepancy between the two standard solutions was less than 0.1 minutes, validating the results and allowing for the progression to the analysis of the solvent. Figures 4, 5, 6, and 7 exhibit the calibration curves for cyclohexane, p-xylene, toluene, and o-xylene, established by plotting peak area against concentration (ppm). All four calibration curves show excellent linearity with R^2 at unity or approaching unity, thereby facilitating the determination of the concentrations of identified and quantified organic compounds, cyclohexane and p-xylene, and toluene and o-xylene (Table 4). For the evaluation of the compound within the solvent sample, characterized by a broader temperature range yet matching the standards' rate, a temperature gradient program named petrol gradient was employed. This choice was made considering the potential presence of additional sample constituents absent in the standards, which may elute later; thus, a higher temperature range is recommended to ensure their identification. The qualitative and quantitative analysis outcomes are succinctly presented in Table 5.

Table 4 Quantification data for analysis of commercial cleaning solvent analysis

	cyclohexane	**p-xylene**	**toluene**	**o-xylene**
Concentration (ppm)	**Peak area**			
0	0	0	0	0
100	950	1414	1083	15331
1000	10861	14982	13891	16409

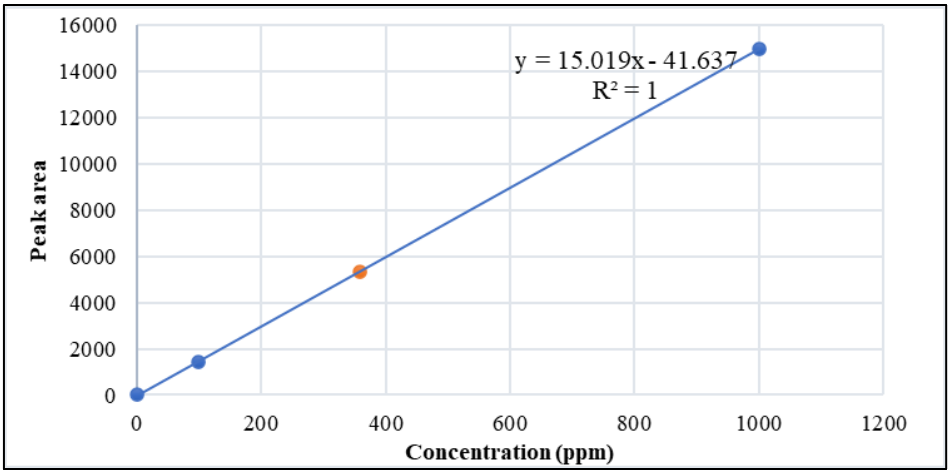

Figure 4 Calibration curve for cyclohexane

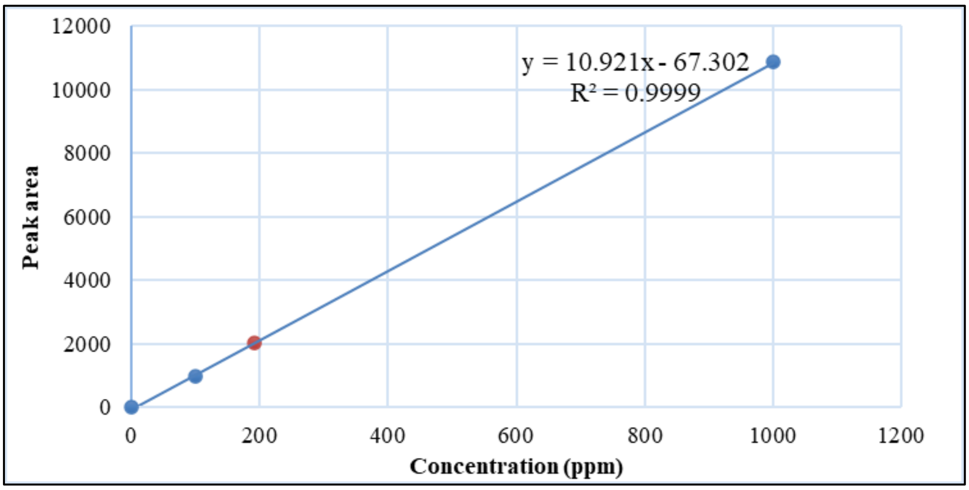

Figure 5 Calibration curve for p-xylene

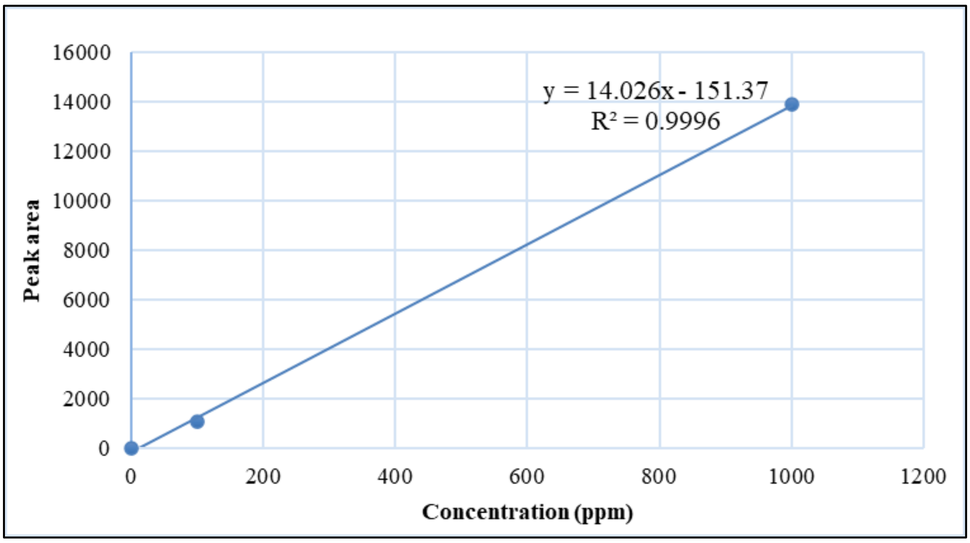

Figure 6 Calibration curve for toluene

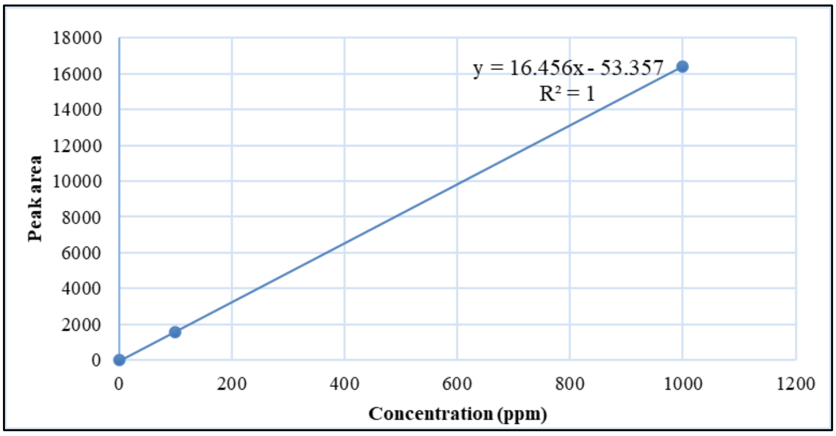

Figure 7 Calibration curve for o-xylene

Table 5 Results of the identified compounds in cleaning solvent

Identified compounds in cleaning solvent	Average peak area	Concentration (ppm)	SD (ppm)
cyclohexane	2035	193	±2.2
p-xylene	5345	359	±5

4. Conclusion

The effect of temperature programming on separations as well as the analysis of different compounds in an unknown sample was successfully investigated using GC-FID. The results obtained illustrate that the efficiency of separation depends greatly on the column temperature, in which the gradient program (40°C to 100°C at 20°C/min) was seen to be a more efficient temperature programming system than the isothermal programs (40°C and 50°C). Improved values for N_{plates}, HETP, and resolution were obtained when the gradient temperature program was used, which means better separation was achieved. Also, the commercial cleaning sample was analyzed qualitatively and quantitatively for the organics present, and the results obtained show that the compounds present are cyclohexane and p-xylene with concentrations of 193 ± 2.2 ppm and 359 ± ppm, respectively. Excellent linearity with correlation coefficient $R^2 = 1.000$ was obtained for all analytes. This study shows that the temperature of the column plays an important role in the separation of compounds using GC, whereby gradient temperature programs ensure all analytes are eluted from the column, and the relatively shallow ramping gives good peak separation. Thus, making it the optimized temperature program for separation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interests.

Statement of informed consent

All authors give their consent to the publication of their data.

Author's contribution

Hauwa Abubakar Rasheed: Conceptualization, investigation, and writing of the original draft. Chizoma Nwakego Adewumi: resources, revising, and editing the manuscript.

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