Analysis of temperature and column variation in gas chromatography to dead time of inert gas and n-alkane homologous series using randomized block design

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Abstract. The dead time behavior from several inert gas and mathematical methods under temperature and column variation have been studied using randomized block design. Dead time of inert gas and mathematical dead time calculated using n-alkane homologous series that measurement with gas chromatography at temperatures of 60, 90 and 120°C and columns of PS-255, OV-11, CPSIL-5CB, HP -5 and HP-INNOWAX were used as a set data in randomized block design. The results showed that temperature variation affects all dead time values, either for inert gas dead time or mathematical method, except for Parcher method. Different column types between capillary and packed column exhibits a significant effect. For capillary column, all dead time values, either for inert gas dead time or mathematical method is not affected by polarity variation, but they are affected by the column length. Meanwhile for packed column, column length does not affect the mathematical dead time of Ne and He gases, but it affects the dead time of O$_2$ and Ar gases. Parcher method exhibits the best result with a robust dead time, without significant effect in statistics by the change of temperature and column.

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1. Introduction
In gas chromatography, temperature and polarity variations are fundamental aspect to obtain the perfect separation on the system. Both parameters affect the degree of interaction between the separated compound and the stationary phase, thus an effective separation time can be achieved. Therefore, one compound has different retention time at different experiment condition. That is why in chemical compound analysis by chromatography; retention time has not been able to provide chemical information from the compounds analyzed. Information is obtained after they have been combined with other methods such as mass spectroscopy[1]. The difference in dead time, the interaction between inert gas (such as Ne, He, O₂ and N₂) and stationary phase does not take place. Hence, no interaction variable that is affected by the change of temperature and polarity in the dead time determination[2]. In case of an isolated system such as chromatography column, the increase of temperature gives an effect against gas pressure, entropy and viscosity[3]. In mobile condition, each compound will have kinetic effect that gives forces on the compound without exception, even for the inert gas. The force causes polarization of the compound and leads to the interaction with the stationary phase. Both thermodynamic and kinetic aspects give the impact on the gas inert flowrate, thus impacts the dead time value.

Besides by injecting gas inert, dead time can also be determined by the mathematical equations using the data of carbon number and retention time from homologous series. Several methods have been introduced for gas chromatography, some of them are statistics [4], iteration [5], non-linear [6], etc. Some literatures have reported the comparison of those methods, reported that there are no methods which give identical dead time of one another in different experimental conditions [7,8]. The different is found in calculated dead time, in several conditions between one method with another, though it is not significant [9,10]. The difference of dead time value is also shown by the measurement of inert gas, either in gas variation or temperature and column variation.

Due to the difference of dead time value, either for inert gas dead time or mathematical method against the change of temperature or column, it is important to study how significant the effect is. This can an additional reference in choosing the robust method against the change of experimental condition. The less the effect of the temperature and column, the better the method. A statistical method has been developed to map a big and heterogen data resulted from different variables of the data source [11], one of which is randomized block design (RBD). The data are mapped into groups which fit against the effect of a variable.

In this research, dead time is determined by several inert gas with mathematical method at several variations of temperature and column, which then will be analyzed with RBD method. The grouping result of temperature and column variables will be analyzed to evaluate the significance of the effect for every method.

2. Methods

2.1. Data collection
The dead time data were collected from the literature of Quintanilla-Lopez et al [8]. The data consist of the dead time of inert gas (Ne, N₂, O₂ and Ar) and mathematical dead time (method of LQG 2, LQG 3 [8], Parcher [12], Dominguez [13] and Guardino [5]) that measurement in temperature and column variations. The temperature variations used are 60, 90, and 120°C and the column variations used are PS-255 (packed column; non polar; 4 m length), OV-11 (packed column; non polar; 2 m length), CPSIL-5CB (capillary column; non polar; 50 m length), HP-5 (capillary column; non polar; 60 m length), HP-INNOWAX (capillary column; polar; 60 m length).

2.2. Randomized block design
Randomized block design (RBD) is a single factorial design by grouping the data into the appropriate group [14]. The grouping is expected to reduce error caused by inhomogeneous experimental units. Analyzed data with RBD is considered to be heterogeneous data, but can be grouped into a factor.
Therefore, data grouping essentially has to follow the characteristic of the data. Linear model of RAK analysis is:

\[ Y_{ij} = \mu + K_j + \tau_i + \epsilon_{ij} \quad \text{with} \quad i = 1, 2, 3 \ldots t \text{ and } j = 1, 2, 3 \ldots k \]  

(1)

Where \( Y_{ij} \) is response variable value of treatment \( i \) on group \( j \), \( \mu \) is general average, \( K_j \) is additional effect caused by group \( j \), \( \tau_i \) is additional effect caused by treatment \( i \) and \( \epsilon_{ij} \) is experimental error caused by treatment \( i \) on group \( j \). The purpose of RBD analysis is to see the effect of the treatment. Hence, RBD hypothesis is as follows:

\[ H_0 : \tau_1 = \tau_2 = \tau_3 = \ldots = \tau_t \]  

(2)

\( H_1 \) is one pair minimum \( \tau_i \neq \tau_{i'} \) where \( i \) and \( i' = 1, 2, 3, \ldots t \).

Hypothesis zero states that there is no difference between \( \tau_1 = \tau_2 = \ldots = \tau_t \) so that, it can be concluded that the treatment does not affect the response variable. In contrary, hypothesis one states that there should be at least one pair of \( \tau_i \neq \tau_j \) or the response variable is found to be affected by the treatment. The calculation of RBD is presented in the table of variance analysis (ANOVA) below.

| Source of variance | Degree of freedom | Sum of Square | Mean Square | F Ratio |
|--------------------|-------------------|---------------|-------------|---------|
| Blocks             | \( b-1 \)         | SSB           | \( \frac{SSB}{b-1} \) | MST \( \frac{MST}{MSE} \) |
| Treatments         | \( k-1 \)         | SST           | \( \frac{SST}{k-1} \) | MSE     |
| Error              | \((b-1)(k-1)\)    | SSE           | MSE         |         |
| Total              | bk-1              | Total SS      | MSE         |         |

where \( b = \) number of block; \( k = \) number of treatment

Sum of Squares Blocks (SSB)

\[ SSB = k \sum_{j=1}^{b} (\bar{Y}_j - \bar{Y})^2 = \sum_{j=1}^{b} \frac{\bar{Y}_j^2}{b} - CM \]  

(3)

Sum of Squares Treatment (SST)

\[ SST = b \sum_{i=1}^{k} (\bar{Y}_i - \bar{Y})^2 = \sum_{i=1}^{k} \frac{\bar{Y}_i^2}{b} - CM \]  

(4)

Sum of Squares Error (SSE)

\[ SSE = Total SS - SSB - SST \]  

(5)

Total of Sum Squares (Total SS)

\[ Total SS = \sum_{i=1}^{k} \sum_{j=1}^{b} (Y_{ij} - \bar{Y})^2 = \sum_{i=1}^{k} \sum_{j=1}^{b} (Y_{ij} - \bar{Y})^2 \]  

(6)

Mean Square of Treatment

\[ MST = \frac{SST}{k-1} \]  

(7)

Mean Square of Error (MSE)
\[
MSE = \frac{SSR}{n-1}
\]  

\[
\hat{Y} = \text{(average of all } n \text{ = bk observations)} = \frac{1}{bk} \sum_{i=1}^{k} \sum_{j=1}^{b} Y_{ij}
\]  

\[
CM = \frac{(total\ observations)^2}{n} = \frac{1}{bk} (\sum_{i=1}^{k} \sum_{j=1}^{b} Y_{ij})^2
\]

Decision to reject \( H_0 \) is taken if \( F_{hit} \) is higher than \( F_{table} \) or \( \alpha \) is higher than \( p \)-value and vice versa. If the decision of the analysis rejects \( H_0 \) (hypothesis zero), then further analysis will be required. Further test is conducted to see the difference between the effect of one treatment with the effect of another one treatment[15].

2.3. Data analysis

The data which have been collected are analyzed with XLSTAT. After the data are input to the software, the analysis was run with randomized blocking design.

3. Results and Discussion

3.1. Test of temperature effect against dead time

The effect of temperature against dead time has been analyzed by using statistical method which is Randomized Blocking Design (RBD). The effect of column temperature variation at 60, 90 and 120°C was analyzed on the dead time determination from Ne, N\(_2\), O\(_2\) and Ar inert gases as well as the dead time from LQG 2, LQG 3, Parcher, Dominguez and Guardino mathematical methods.

| Response Variable | F calculation | P value |
|-------------------|---------------|---------|
| Ne                | 28.82**       | 0.000   |
| N\(_2\)           | 6.57          | 0.031   |
| O\(_2\)           | 27.62*        | 0.001   |
| Ar                | 29.27*        | 0.001   |

Information: * significantly different at significance degree of 0.05  
** significantly different at significance degree of 001

| Response Variable | F calculation | P value |
|-------------------|---------------|---------|
| LQG2              | 56.49**       | 0.000   |
| LQG3              | 53.98**       | 0.000   |
| Parcher           | 1.88          | 0.222   |
| Dominguez         | 109.89**      | 0.000   |
| Guardino          | 95.82**       | 0.000   |

Information: ** significantly different at significance degree of 0.01

Table 2 and 3 exhibit the presence of temperature effect against the dead time. Hypothesis \( H_0 \) is rejected if \( p \)-value is lower than 0.05. This means the temperature has an effect against the dead time value. The analysis result of ANOVA RDB shows that only dead time from Parcher method that is not
affected by the change of temperature, where the p-value is 0.222 at the trust degree of 95%, meanwhile other methods show the effect of temperature.

| Response variable | Group | Temperature (°C) |
|-------------------|-------|-----------------|
|                   |       | 60   | 90   | 120  |
| Ne                | 5     | 3.65a | 3.93b | 4.21c |
| N<sub>2</sub>     | 5     | 3.75b | 4.03b | 4.17b |
| O<sub>2</sub>     | 5     | 3.77b | 4.03b | 4.25c |
| Ar                | 5     | 3.76b | 4.04b | 4.25c |

Information: the similar alphabet is given to indicate the insignificant different at α = 0.05

The RDB analysis was proven by Duncan test. Duncan test is used to identify which variation gives unsimilar average between 60, 90 and 120°C. The change of temperature is found to not give an effect against the dead time value if the result of Duncan test labeled with the same alphabet between one variation and the others. The result of Duncan test shows that dead time determined by Parcher method is observed to have no effect of temperature at all temperature variations. Either at 60, 90 and 120°C, all the test results are given label a. Dead time from N<sub>2</sub> gas shows the effect of temperature which on takes place at 60°C, meanwhile at 90 and 120°C there is no indication of an effect against the dead time value where both results of Duncan test are given label b.

| Response variable | Group | Temperature (°C) |
|-------------------|-------|-----------------|
|                   |       | 60   | 90   | 120  |
| LQG 2             | 5     | 3.64a | 3.91b | 4.10c |
| LQG 3             | 5     | 3.64a | 3.91b | 4.14c |
| Parcher           | 5     | 3.63a | 3.65a | 4.30a |
| Dominguez         | 5     | 3.53a | 3.83b | 4.06c |
| Guardino          | 5     | 3.53a | 3.83b | 4.05c |

Information: the similar alphabet is given to indicate the insignificant different at α = 0.05

| Response Variable | F calculation | P value |
|-------------------|---------------|---------|
| Ne                | 636.061**     | 0.000   |
| N<sub>2</sub>     | 429.19**      | 0.000   |
| O<sub>2</sub>     | 1282.34**     | 0.000   |
| Ar                | 1342.33**     | 0.000   |

Information: **significantly different at significance degree of 0.01

3.2. Test of column effect against dead time

RDB method is used to analyzed the column effect against the dead time value. Column variations used are 2(PS-255), 4(OV-11), 7(CPSIL-5CB), 8(HP-5) and 9(HP-INNOWAX), where the response variable include the dead time of inert gas (Ne, N<sub>2</sub>, O<sub>2</sub>, Ar) and the dead time calculated with LQG 2,
LQG 3, Parcher, Dominguez and Guardino equations. In the inert gas dead time studies, column CPSIL-5CB is not used, due to the incomplete data from the literature.

The result of ANOVA RDB analysis shows that column variation gives effect which is significant against all the dead time used, either inert gas or mathematical method dead time. This is shown by the obtain p-value for all dead time methods is zero.

The result of Duncan test for inert gas dead time shows that the variation of two capillary-type columns does not show any effect against all inert gas dead time. This is shown by the same alphabetical labels between the analysis result at HP-5 and HP-Innowax columns. Meanwhile, the variation of two packed-type columns (PS-255 and OV-11) shows the effect against the dead time of O2 and Ar, yet does not show an effect to the dead time of Ne and N2. These results indicate that at capillary column, polarity variation does not affect the dead time value of inert gases. Meanwhile at packed column, column length does not affect the dead time value of Ne and N2 gases, though it affects the dead time of O2 and Ar.

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### Table 7. F calculation and P value from ANOVA RDB table for the column treatment per response variable for mathematical calculated dead time.

| Response Variable | F calculation | P value |
|-------------------|--------------|---------|
| LQG2              | 1755.992**   | 0.000   |
| LQG3              | 1481.385**   | 0.000   |
| Parcher           | 25.527**     | 0.000   |
| Dominguez         | 2964.476**   | 0.000   |
| Guardino          | 2669.349**   | 0.000   |

Information: **significantly different at significance degree of 0.01

### Table 8. The result of advanced Duncan test for column treatment for inert gas dead time.

| Response Variable | Group | Column |
|-------------------|-------|--------|
|                   |       | PS-255 | OV-11 | HP-5 | HP-INNOWAX |
| Ne                | 3     | 2.42^a | 2.23^a | 5.71^c | 5.66^c |
| N2                | 3     | 2.33^a | 2.22^a | 5.71^b | 5.66^b |
| O2                | 3     | 2.46^b | 2.23^a | 5.71^c | 5.66^c |
| Ar                | 3     | 2.47^b | 2.23^a | 5.71^c | 5.66^c |

Information: the treatment with similar alphabet is not significantly different at α = 0.05

### Table 9. The result of advanced Duncan test for column treatment for mathematical methods.

| Response Variable | Group | Column |
|-------------------|-------|--------|
|                   |       | PS-255 | OV-11 | CPSIL-5CB | HP-5 | HP-INNOWAX |
| LQG2              | 3     | 2.37^a | 2.24^a | 3.50^b | 5.70^c | 5.60^c |
| LQG3              | 3     | 2.37^a | 2.24^a | 3.50^b | 5.70^c | 5.66^c |
| Parcher           | 3     | 2.32^a | 2.23^a | 4.16^b | 5.70^c | 5.50^c |
| Dominguez         | 3     | 2.11^a | 2.11^a | 3.47^b | 5.68^c | 5.65^c |
| Guardino          | 3     | 2.51^a | 2.11^a | 3.47^b | 5.67^c | 5.65^c |

Information: the treatment with similar alphabet is not significantly different at α = 0.05
The result of Duncan test for the dead time obtained from mathematical method shows that the variation of two packed-type columns does not give an effect against all the dead time. This is shown by the same alphabetical labels among the analysis results at the used columns (PS-255 and OV-11). Meanwhile the variation of three capillary-type columns (CPSIL-5CB, HP-5 dan HP-Innowax) shows that all the dead time obtained through mathematical methods is affected by column CPSIL-5CB against two columns (HP-5 and HP-Innowax). For the variation of two capillary column (HP-5 and HP-Innowax), there is no effect shown against all the mathematical dead time. This shows that at capillary column, polarity variation does not give an effect against the dead time values obtained through mathematical methods, yet it is affected by the column length variation. In contrary, at packed column, column length does not affect all dead time values of the mathematical methods.

4. Conclusion
Temperature variation affects all dead time values, either the dead time values determined by inert gases or mathematical approaches, except for Parcher method. Different types of column, the capillary and the packed columns, contribute to the significant effect. At capillary column, all dead time values obtained from inert gases or mathematical methods, are not affected by polarity variation, yet they are affected by the column length. Meanwhile at packed column, column length does not affect the dead time from the mathematical methods, Ne and He gases, yet affects the dead time values of O₂ and Ar. Parcher method exhibits the best result with robust dead time values without being statistically significantly affected by the change of temperature and columns.

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