Dead time determination of 2-alkanone homologues series using methanol/water eluent in high performance liquid chromatography system by indirect method

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Abstract. The calculations of the dead time have been done in High Performance Liquid Chromatography System using spreadsheet method. The dead times produced by spreadsheet method were compared to the dead times of iteration method. The dead time were calculated using retention time and carbon number of 2-alkanone that eluted in several eluent composition of methanol/water. The dead time value generated by spreadsheet method shows the dead time value with equally good criteria to iteration method in generating the dead time value as low as possible, where the standard deviation of dead time value against the change of eluent composition is relatively low and the plot linearity of ln(κ’) vs nC is shown to be good.

1. Introduction
Chromatography parameters are classified into two groups, primary and secondary parameters. Primary parameter is obtained directly from chromatography instrument measurement, but secondary parameter is obtained from mathematic calculation using primary parameter data. Only retention time and dead time that are classified as primary parameter, meanwhile other parameters are secondary one [1]. Retention time is often used in chemical compound identification [2], but dead time is only used when secondary parameter is required. Secondary parameters hold an important role to emphasize psycho-chemistry [3] and thermodynamic [4] information of the analyzed compound. Therefore, the accuracy of retention time and dead time calculation is very important in determining the validity of the secondary parameters such as adjusted retention time, capacity factor [5], and retention index [6,7].

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Dead time is obtained through the retention time determination of mobile phase, which include the retention time of inert gas in gas chromatography and the retention time of eluent in liquid chromatography. Within the routine analysis in a laboratory, the determination of dead time shows several serious challenges. In gas chromatography, some gases cannot be detected by flame ionization detector (FID) [8]. Moreover, during the initial elution condition, noise intervention and difficulties in identifying gas peak are still commonly found [9]. In liquid chromatography, dead time is determined by using identifying marker such as isotopes, radioactives, ionic species and anions added in the mobile phase. Nevertheless, those markers are dangerous and toxic to environment [10]. Furthermore, dead time value from the marker method is not robust against the marker variation used and the change of experimental condition [11]. Dead time value of marker method is still affected by the composition of the used solution [12].

Alternative method to determine a dead time is by mathematical algorithm approach. Some popular methods that have been practiced in gas chromatography is graphic [13], flexible simplex [14], statistics [15,16], iteration [17], multiparametric [18], non-linier [19], and spreadsheet [20] methods. In liquid chromatography, calculation algorithms of dead time have also been introduced, some of which are linearization [10,21,22], graphic [23] and nonlinear [24] methods. Those methods are applied by using the correlation between retention time and carbon number of homologous series determined in isothermal condition in gas chromatography or in isocratic condition in liquid chromatography [17,25–27].

To this date, there is no agreement on the standard definition and method of determining the dead time, especially in liquid chromatography. Some principles are being the standard to claim the quality of the dead time, which include accuracy of fit (the less S/N, the better the method used) [28,29], the method which results in lowest dead time is claimed to have best method [30], correlation linearity between natural logarithm of capacity factor (ln k’) and carbon number (nC), and deviation standard between dead time against the change of solution composition has to be low [31]. Liquid chromatography system is more complex than gas chromatography causing the dead time study with marker method still find many challenges due to the absence of the compound that actually detained in liquid chromatography column, even the solution is also detained in the column [10]. Therefore, the mathematical method study to obtain the best method is the best solution for liquid chromatography problem.

Several researches show that iteration method still become the best one in dead time calculation, either in gas or liquid chromatography. Smith et al [28] reported that, in gas chromatography, flexible simplex [14], iteration [17] and statistics [16] methods give the most accurate dead time, with iteration method [17] also give the quickest calculation, compared to statistics [15,16], exact calculator [32], dual linear regression of quadratic and cubic fit [33], and single linear regression [34] methods. A research in gas chromatography by Quintanilla-Lopez et al (1997) [35] shows that iteration method give lower dead time than exact calculator [32], linearisation [36], and LQG [35] methods. It means that iteration method gives the best dead time compared to others.

Several methods to calculate dead time in gas chromatography, such as iteration, multiparametric and statistics [4], have been successfully applied well in liquid chromatography. Idroes (2005) [37] reported that iteration method give more accurate dead time than linearisation method. This conclusion is also strengthened by the result which is in agreement with the dead time calculated from several homologous series (n-alkane, alkylbenzene [38], 2-alkanone [39], and alkylarylketone [40]) at some eluent compositions. This report is interesting, because of the fact that linearization method is initially
developed for liquid chromatography system, meanwhile iteration method even has been developed for gas chromatography.

Braithwaite and Cooper (1993) [20] introduced spreadsheet method for dead time determination in gas chromatography, where it shows an acceptable degree of accuracy with reference to flexible simplex method. This method also gives quick calculation, even quicker than the iteration method (Braithwaite and Cooper, 1993) [20]. In this research, spreadsheet method is applied for dead time calculation at Reverse Phase High Performance Liquid Chromatography (RP-HPLC). The calculation is conducted with the data of homologous series of 2-alkanone at several methanol/water eluent compositions. The determination accuracy of the spreadsheet method is observed by the comparison of dead time value against the iterative method, linearity principle and the effect of eluent composition.

2. Methods and data

2.1. Material
The homologous series used were 2-alkanone from Riedel de Haehn. The eluent used were methanol and water for HPLC grade from Merck.

2.2. Instrumental
The column was Lichrospher 100 RP-18 (Merck) with 5 µm diameter and 125 x 4 mm. Pump was Bischoff model 2200 (serial 903). Detector was RI-detector model ERC-7510. Recorder: HP-3394, Hewlett-Packard.

2.3. Procedure
The solvents (variations of methanol/water were set at 80/20, 70/30, 60/40 and 50/50) were degassed approximately 10 min with Helium (He). The HPLC equipment with particular refractive-index detector has been pre-run till equilibrium condition before injection. The column was adjusted in water jacket at 30 °C. Solvent-reservoir was put in water jacket and kept at 30 °C constantly. Flow-rate: 1.0 mL/min. The samples (2-alkanone homologoues series) were injected.

2.4. Spreadsheet Method
This experiment used spreadsheet macro which was running by keystroke initiation, based on the Spreadsheet method developed by Braithwaite and Cooper [20]. The initial arbitrary dead time ($t_{R1}$) was used to calculate the adjusted retention times. Plot of the logarithm of the adjusted retention times versus the carbon numbers was made to produce the correlation coefficients. The iterative process was carried out by decrement of the dead time ($dt_0$) until the optimal correlation coefficient was reached to produce the most accurate dead time.

2.5. Iteration Method
The iteration method was developed by Guardino et al [17]. An initial dead time was estimated to calculate the adjusted retention time. Plot of the semi-logarithm using a least-squares fit was made to determine slope and intercept. The process was continued with iteratives by minimizing the sum of squares of the difference between the known and estimated index retention values until the best dead time value, slope and intercept were determined.

3. Result and discussion
In this research, spreadsheet method basically is developed to determine dead time in GC, then used to determine dead time in RP-HPLC system. The determination of dead time is conducted using retention data from homologous series of 2-alkanone at four different methanol/aqueous eluent compositions. As a comparison, the determination of dead time with iteration method is also conducted (Table 1). The result shows the dead time of both methods for all eluent composition is the same. This means the
spreadsheet method fulfills the similarly good criteria with iteration method in determining the dead time as low as possible [30].

Table 1. Mathematical dead time calculated by spreadsheet and iterative methods using 2-alkanone homologous series data eluted by different composition of methanol/water eluents.

| Methods   | Eluent Composition (Methanol/Water) | SD   | Error (%) |
|-----------|-------------------------------------|------|-----------|
|           | 80/20 | 70/30 | 60/40 | 50/50 |      |      |
| Spreadsheet| 1.136 | 1.214 | 1.187 | 1.187 | 0.033 | 3.259 |
| Iteration | 1.136 | 1.214 | 1.186 | 1.186 | 0.032 | 3.247 |

When it goes through chromatography column, a component will undergo two phases, which are static phase and dynamic phase respectively and continuously within the column. The static phase is a condition where the component is restrained in the column by the interaction with stationary phase, meanwhile the dynamic phase is a condition where the component is moving within the mobile phase without being restrained by the stationary phase. In GC system, the mobile phase is inert and assumed to have no significant interaction with the stationary phase. Therefore, the time needed by the GC mobile phase or other inert components in GC column can be correlated as a dead time. In contrast with LC system, mobile phase has an interaction with stationary phase and restrained in the column. The compound determination without interaction with the stationary phase (marker compound) will also be restrained within the mobile phase. Fundamental variation of eluent composition in LC analysis gives additional effect towards the dead time determination. The condition causes marker method (direct method in LC system) to be unideal to be applied. The application of mathematical methods are expected to be the solution to reduce the non ideal aspect of marker method.

Table 2. Natural logarithm of capacity factor from 2-alkanone homologous series calculated using mathematical dead time in each eluent composition.

| $n_c$ | $\ln(k')$ |
|-------|-----------|
|       | Spreadsheet Dead Time | Iteration Dead Time |
|       | 80/20 | 70/30 | 60/40 | 50/50 | 80/20 | 70/30 | 60/40 | 50/50 |
| 3     | -      | -1.766 | -1.546 | -   | -    | -1.760 | -1.541 |
| 4     | -      | -1.481 | -1.158 | -0.764 | -   | -1.481 | -1.154 | -0.761 |
| 5     | -      | -1.001 | -0.482 | 0.013 | -   | -1.001 | -0.480 | 0.015 |
| 6     | -0.833 | -0.448 | 0.153 | 0.770 | -0.833 | -0.448 | 0.154 | 0.771 |
| 7     | -0.437 | 0.088 | 0.793 | 1.545 | -0.437 | 0.088 | 0.794 | 1.546 |
| 8     | -0.020 | 0.620 | 1.431 | 2.324 | -0.020 | 0.620 | 1.432 | 2.325 |
| 9     | 0.382 | 1.143 | 2.071 | 3.105 | 0.382 | 1.143 | 2.072 | 3.106 |
| 10    | 0.786 | 1.663 | 2.709 | -   | 0.786 | 1.663 | 2.710 | -   |
| 11    | 1.190 | 2.187 | -   | -   | 1.190 | 2.187 | -   | -   |
| 12    | 1.598 | 2.707 | -   | -   | 1.598 | 2.707 | -   | -   |
Based on the unrestrained principle, a good dead time cannot be affected by the change of eluent composition [31]. Because of the impossibility, then the change effect of eluent composition is accepted within low standard deviations. Therefore, method resulting a dead time with low standard deviations against the change of eluent composition is suggested to be best method. The calculation result, either from spreadsheet or iteration method, show the effect of eluent composition change against the dead time value with relatively low standard deviation from 3.259 % and 3.247% for spreadsheet and iteration methods (Table 1), respectively. This result shows that spreadsheet method meets the criteria that are equally good to iteration method in term of dead time determination with low standard deviation.

A good dead time will give the accurate determination of secondary retention parameter as in adjusted retention time ($t'_R$) and capacity factor ($k'$). Those to secondary parameters are intercorrelated and important in giving psycho-chemistry [3] and thermodynamic [4] information in separation efficiency or retention index determination [6]. During the determinations of adjusted retention time, the dead time corrects the time used by a restrained component within the dynamic phase until the true restrained/interaction time of the compound gains the stationary phase can be determined. In the determination of capacity factor, dead time determines the comparative scale between the used time by the restrained compound to be in the static phase and dynamic phase. This condition allows the formation of systematic correlation between psycho-chemistry and thermodynamic parameters ($t'_R$ or $k'$) against the identity of the restrained compound (carbon number ($n_C$)).

Ideally, there is a correlation between natural logarithm of capacity factor towards carbon number. Because carbon number is a constant variable, thus the linearity will be determined by the accuracy of capacity factor, in this case the accuracy of dead time calculation. More accurate the calculation of of dead time value, the more linear the relation between ln($k'$) vs $n_C$ [31]. Therefore, correlation linearity of ln($k'$) vs $n_C$ can be used as a standard to justify the accuracy of the determined dead time as well as the quality of the dead time determination method.

Natural logarithm of capacity factor of homologous series of 2-alkanone has been calculated by spreadsheet and iteration methods at some eluent composition variations (Table 2). The result shows that ln($k'$) value of both dead time calculation methods at all eluent composition is relatively the same. The correlation of ln($k'$) vs $n_C$ has been plotted on cartesian plane, either for spreadsheet or iteration methods (Figure 1). Those plots also show regression parameters such as slope and intercept, which the results from both methods are relatively the same (Table 3). This similarity has a correlation with time values of the both methods, which are relatively the same.
Table 3. Parameters of linear regression of natural logarithm capacity factor versus carbon number.

| Methods       | Regression Parameters | Eluent Composition |
|---------------|-----------------------|--------------------|
|               |                       | 80/20  | 70/30  | 60/40  | 50/50  |
| Spreadsheet   | Slope                 | 0.40539 | 0.52660 | 0.64101 | 0.77353 |
|               | Sdv Slope             | 0.00077 | 0.00187 | 0.00179 | 0.00140 |
|               | Intercept             | -3.26752 | -3.60408 | -3.69774 | -3.86306 |
|               | Sdv Intercept         | 0.00706 | 0.01574 | 0.01232 | 0.00887 |
|               | Sdv Regression        | 0.00405 | 0.01450 | 0.01159 | 0.00742 |
|               | Coefficient Determination | 0.99998 | 0.99991 | 0.99995 | 0.99998 |
| Iteration     | Coefficient Determination | 0.99998 | 0.99991 | 0.99995 | 0.99998 |
|               | Sdv Regression        | 0.00405 | 0.01450 | 0.01159 | 0.00744 |
|               | Slope                 | 0.40539 | 0.52660 | 0.64040 | 0.77297 |
|               | Sdv Slope             | 0.00077 | 0.00187 | 0.00179 | 0.00141 |
|               | Intercept             | -3.26752 | -3.60408 | -3.69167 | -3.85779 |
|               | Sdv Intercept         | 0.00706 | 0.01574 | 0.01233 | 0.00889 |

Linearity of a correlation between $\ln(k')$ vs $n_C$ can be observed by the correlation coefficient. The correlation coefficient of both methods at all eluent composition shows a highly linear value (Table 3) up to 4 decimals, which is 0.9999. Coefficient correlation value of both methods at all eluent compositions is exactly the same. The best coefficient correlation is given by eluent composition of 80/20 and 50/50. This result shows that the spreadsheet method fulfill the similarly criteria as the iteration method in giving the result of good linearity of $\ln(k')$ vs $n_C$ plot.

4. Conclusion
Dead time determination using spreadsheet has been successfully applied in HPLC system at different eluent compositions. Spreadsheet method is capable to give dead time value with the criteria that are as good as iteration method, where the dead time is given in the lowest possible value. The deviation standard of the dead time value against the change of eluent composition is relatively low and very good linearity of $\ln(k')$ vs $n_C$ plot.

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