The influence of temperatures, polarity, modifier and pressure to retention index in supercritical fluid chromatography: A review

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Abstract. Changes in analysis parameters affect the retention index calculation on all chromatography systems. In Supercritical Fluid Chromatography, the analysis parameters are more than Gas Chromatography or Liquid Chromatography. In this study, the effect of all those parameters were presented based on the research that has developed to date. The data and the information were collected from the previous published articles. The changes in the analysis parameters which are temperature, polarity, modifier, and pressure on SFC affected the retention index calculation, but the significant levels were different. The pressure change has the least significant effect for both polar and non-polar analytes. The temperature and stationary phase polarity changes have insignificant effects for non-polar analyte, but significant for polar one. Meanwhile, the modifier change have a very significant effect, both for polar and non-polar analytes.

1. Introduction

Retention index studies on chromatography have shown great progress. The retention index calculation has been able to provide the accurate and reliable results. This success has changed the function of chromatography from being just a separation method to the chemical profile identification of the analytes [1].

In classical analysis, chromatography yields the separation information through the retention time. The number of retention time is proportional to the number of analytes contained in the sample. However, the retention time cannot provide the information of the chemical profile of the analytes. This is due to the dependence of the retention time to the analysis condition. If the analysis parameters
such as temperature and column polarity are changed, the retention times also change [2]. Therefore, to determine the chemical profile of the analyte, it need to use either additional methods or instruments. If the analyte profiles is already known, identification is done by measuring the retention time of the standard compound [3]. However, if the analyte is a new or unknown compounds, a tandem instrument is carried out where each retention time is identified by the tandem, such as mass spectrometry [4–9], infrared spectroscopy [10], or nuclear magnetic resonance [11].

In modern analysis, the retention time can also be converted into a retention index by utilizing the dead time and the carbon number of the standard compound. The three retention parameters were applied to a mathematical algorithm introduced by Erwin Kovats in 1958 [12,13]. The retention index is robust against the changes of the analysis parameters. Although the retention of certain analyte varies, the retention index calculation always produce a relatively fixed value [14]. The retention index is related to the analyte carbon number. The carbon number is related to the analyte polarity. Therefore, if the retention index can be calculated, the carbon number and polarity of the analyte can be predicted [15].

The main challenges in determining the retention index are the accuracy of a dead time determination and a selectivity of the separation. The dead time is calculated either by measuring an inert gas in the chromatography column or by using a homologous series method [16]. With algorithms, the retention times of the homologous series are converted into the dead time [17,18]. As chromatography measurement products, both methods are always challenged by various analysis parameters [19]. As with the dead time, the selectivity of separation also plays an important role because it determines the accuracy of determining the retention time. In fact, this last one is only achieved at an optimum level through the determination of the suitable analysis parameters [20]. Therefore, the accuracy of the retention index is indirectly related to the analysis parameters.

The parameters which affect the retention index calculation in Gas Chromatography (GC) include polarity, temperature and column length [21]. In Liquid chromatography (LC) and supercritical fluid Chromatography (SFC), the number of these parameters increases, namely solvent composition, modifier, homologous series type and pressure [22,23]. The accuracy issue of the retention index calculation in GC system was mostly solved, where homologous series methods such as iteration, spreadsheets and statistics can produce the accurate dead time against the changes of analysis parameters [24]. In fact, this retention index has been applied in the identification of organic compounds [25–28].

In LC and SFC, the accuracy of the retention index calculation is weaker than in GC. This is because the analysis parameters is more complex. This problem has not been fully resolved neither by dead time algorithm nor by analysis method [29]. However, the study of retention index in LC is more developed than SFC. Calculations of both the retention index and the dead time in LC have several improvement and become closer to accurate [30,31]. In addition, the study in SFC itself has been slower to run. There were only a few related publications that discussed in this regard.

In this study, a discussion were carried out related to retention index studies on SFC. Various analysis parameters, which are temperature, polarity, modifier and pressure, affected the retention index calculation were described in detail. So that researchers can find out the latest developments in SFC. So that, it can facilitate the development of further research.

2. The effect of temperature and polarity on the retention index calculation
Temperature greatly determines the selectivity of the separation. It affects the strength of the interaction between the analyte and a stationary phase. This causes a shift in an elution ability to the marker measurement as well as the homologous series separation in the dead time determination [32].

The studies of 5 marker types representing 5 different polarity, as Table 1 illustrates, showed both in polar and non-polar columns fluoro cyclic marker provided the most constant dead times against the temperature changes. This marker has the lowest affinity compared to other ones in all column polarity types. The same thing is also shown in the separation of the n-alkanes homologous series under the same conditions. Both the fluoro-cyclic marker and the n-alkane homologous series have the same
constant properties (in terms of separation density) [33]. Therefore, it can be stated that both compounds are assumed to represent the most suitable standard in determining the dead time for the retention index calculation purpose.

| Compound               | Characteristics   |
|------------------------|-------------------|
| Anthracene             | Relatively polar  |
| 1,10-Decanediol        | Polar             |
| Methyl 3,5 dinitrobenzoate | Strongly polar   |
| Methyl myristate       | Relatively non polar |
| Fluoro cyclic          | Non polar         |
| n-Alkanes              | Strongly non polar |

In determining the retention index, the n-alkane homologous series still showed the lowest effect on the changes in temperature and column polarity compared to marker [34]. Among the five markers, methyl myristate dead time produced the most constant retention index compared to others. This retention index increased insignificantly with increasing the polarity. This corresponded to the non-polar character of the strong alkyl and the weak polar one it owned. The temperature changes tested for all column polarity did not even have the effect to the retention index calculation from this compound [33].

The significant effect was shown in the retention index changes calculated by anthracene and methyl 3,3 dinitrobenzoate dead time. There is a significant change against the increase of temperature and column polarity. This was attributed to the polar character possessed by the phi bonds in aromatic rings [33].

This review concluded that the non-polar compounds for dead time calculation had the least significant effect to Retention index calculation against the changes of temperature and column polarity. Therefore, this compound type can be used as a reference for dead time and retention index studies in SFC.

3. The effect of modifier on the retention index calculation

In SFC, a modifier is added into the mobile phase to increase the analyte separation ability in the sample [35]. In some routine SFC analysis, several classes of compounds such as barbiturates, separated by a CO$_2$ mobile phase and a Polystriyrene-divinylbenzene (PS-DVB) column, showed very poor separation. Even with the use of the Octadecyl-silica (ODS) column, the barbitures were completely inseparable [36]. Therefore, a modifier such as methanol is applied to the mobile phase. In the suitable ratio of the mobile phase: modifier composition, the separation are better.

The addition of methanol and acetonitrile modifiers into the CO$_2$ mobile phase caused the change of the retention index. The retention index of the n-alkane and alkylarylketones homologous series, the changes was not very significant, but for the test substance is the otherwise [36].

The study of Barbiturates’ retention index by using alkyl aryl ketones homologous series, CO$_2$ mobile phase, methanol modifier and PS-DVB column showed the significant changes in capacity factor and retention index. The presence of methanol in the mobile phase changes the interaction pattern between each analyte to both the mobile and stationary phases. The changes in modifiers and their composition ratios led to a significant difference in the retention of the relative unrelated compounds between the alkyl aryl ketones homologous series and barbiturates [36].

An alternative method to treat the significant effect of modifier, adjusting the pH on the mobile phase: modifier composition is necessary. Setting the pH of the composition appropriately minimized the retention index changes caused by modifier. However, the calculated retention index obtained wasn’t categorized as constant yet. It mean that the effect of the modifier is still significant [36]. This condition indicates that in the laboratory analysis, the purposes of separation effectiveness and the retention index calculation cannot be done simultaneously.
4. The effect of pressure on the retention index calculation

Pressure is a unique parameter which only exists in SFC. This is related to the use of the supercritical mobile phase which is a function of temperature and pressure. Pressure is very difficult to determine accurately from one analysis to another. Even if the equipment is equipped with standard gauges, the average pressure in the column highly depend on both the column resistance to the mobile phase flow and the pressure exerted by the pump [23].

Retention index studies of several non-polar and polar compounds showed insignificant effect to the pressure changes. However, the changes between non-polar and polar analytes are very different. For non-polar analytes, the increase in pressure causes a decrease in the retention index, whereas for polar analytes, the increase in pressure causes the increase one [23].

5. Last reports

Recent research in the last two decades regarding the retention index in SFC is very difficult to find. Most of the research related to this one was published in the 80-90s and the numbers were minimal. In this section, several studies of retention index in SFC are presented in Table 2 to obtain an overview of the developments which have been achieved by the researchers. It is hoped that this will become a reference in continuing the research.

| Years | Objectives                                                                 | Findings                                                                                          | Ref  |
|-------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|------|
| 1988  | The effect of changes in temperature, pressure and modifiers on the retention index of the n-alkanes, alkyl aryl ketones and alkyl benzene homologous series using the non-polar PS-DVB column | There was the insignificant effect in the retention index of the n-alkanes and alkyl aryl ketones homologous series to the changes in temperature, pressure and the addition of less than 15% organic modifier. | [34] |
| 1989  | The effect of methanol modifier on the retention index calculation of benzodiazepines using the alkyl aryl ketones homologous series | There was a significant decrease in the retention index of benzodiazepines with the increase in the percentage of modifiers | [35] |
| 1989  | The effect of methanol modifier on the retention index calculation of the Barbiturates using the alkyl aryl ketones homologous series | There was a significant decrease in the retention index of barbiturates with the increase in the percentage of modifier | [36] |
| 1990  | The effect of column polarity on the retention index calculation of the Poly-cyclic aromatic hydrocarbon (PAH) | There was an insignificant effect to retention index of PAH against the column polarity variations | [37] |
| 1990  | The effect of the pressure and modifier to retention index calculation of alkyl aryl ketones, alkyl benzenes and test substances using ODS column | The change in pressure caused insignificant changes in the retention index of both the homologous series and the test substances. There was only the retention index of alkyl aryl ketone which was not significantly affected by the addition of modifier, whereas others were otherwise. | [23] |
| 1991  | The comparison of the retention index calculation of aliphatic and cyclic compounds in SFC and GC | The retention index of cyclic compounds is more similar to reference than aliphatic ones | [38] |
1992 The n-alkyl phenones homologous series were applied in the retention index calculation of secondary metabolite from Fusarium mycotoxins separated by capillary and packed column.

1992 The effect of temperature and pressure on the retention index calculation at various temperature and polarity.

1993 The effect of temperature and pressure on the retention index calculation at various temperature and polarity.

1999 The effect of the density change of the CO$_2$ mobile phase on the retention index calculation of cyclic and aliphatic compounds.

6. Conclusion

The changes in the analysis parameters of temperature, polarity, modifier, and pressure have the effect to the retention index calculation in SFC, but differ in the level of significance. The pressure change has the least significant effect for both polar and non-polar analytes. The changes in temperature and polarity have insignificant effects for non-polar analytes, but significant for polar ones. Meanwhile, modifier changes have a very significant effect, both for polar and non-polar analytes.

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