ALKYLARYLKETONE HOMOLOGOUS SERIES FOR DETERMINATION OF KOVATS RETENTION INDICES WITH RP-HPLC USING ACETONITRILE/WATER SYSTEM

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Received October 6, 2009; Accepted January 20, 2010

ABSTRACT

Some factors such as the changes of the stationary phase, temperature, pH-value, mobile-phase composition and flow rate play a crucial role in effecting the sensitivity of retention times in high performance liquid chromatography (HPLC) system. Utilizing a retention index system is one of the methods to minimize those effects. Besides the mentioned factors, dead-time influences on determining the retention index as well. In comparison with Gas Chromatography (GC), the retention Index determination method in HPLC is still widely discussed, due to the difficulty of utilizing n-alkane as standard. In addition, the solutes in HPLC interact with the mobile-phase, thus the retention behavior also depend on the mobile-phase. Actually, It is difficult to use n-alkanes in HPLC as standards in case of some considerable problems, due to they are very non polar but also large retention times which lack of chromophores. Therefore, using n-alkane in routine analysis could be inconvenient. In comparison with n-alkanes, the alkylarylketones homologous series are stable compounds, commercially available and easily detected by a UV detector. This paper introduces Determination of Kovats Retention Index in the HPLC using Alkylarylketone homologous series and then is connected with n-alkane as a frame of reference. Steroids were used as test substance for calculating Kovats retention index values in acetonitrile/water system.

Keywords: *Kovats Retention Index, RP- HPLC, n-alkane, alkylarylketone*

INTRODUCTION

It is not advisable to use retention times to characterize the retention behavior, due to its bad reproducibility. Many factors influence retention time in RP-HPLC, the most important factors are the following: temperature, pH value, mobile phase composition, column material, column age and flow-rate. Among retention parameters such as retention time (t_R), capacity factor (k´) and retention index (RI) show that retention index is more reproducible and more robust than t_R and k´ [1-2]. The dead time (t_0) calculation is needed to determine the mentioned retention parameters. There is no possibility to define a unified dead time since it depends on the chromatographic system and the method how dead time is calculated [3]. In this research, the dead time is calculated by applying homologous series (n-alkane) according to the iteration method of Guardino [4].

n-alkane is suggested to be used as retention index scale in HPLC, due to the results directly comparable with retention data from GC [5]. However, it is not discussed here how to compare the retention data based on n-alkane between GC and HPLC system. In contrast to GC in HPLC solutes also interact with the mobile phase, thus the retention data also depend on the mobile phase. It is not easy to use n-alkane in HPLC as standards in case of two considerable problems. Firstly, they are very non polar, hence in most reversed phase system they show large retention times which restricts their application. Moreover, an additional refractive index detector is needed due to the lack of chromophores. Therefore using n-alkanes in routine analysis is problematic. In comparison with n-alkanes, the alkylarylketone has the advantage that they can be detected spectroscopically. In addition, these homologous series are stable compounds and are commercially available. They have a high UV absorbance at the wavelength of 254 nm, and thus are easily detected by a UV detector [6]. This paper reports Kovats Retention Index Determination in the HPLC by using alkylarylketone homologous series and then is connected with n-alkane as a frame of reference. Steroids were used as test substance for determining absolute retention index values in acetonitrile/water system.

EXPERIMENTAL SECTION

Materials

n-alkane (Aldrich) and alkylarylketone (Riedel de Haehn) substances were used as homologous series, and a solvent system of acetonitrile/water (ACN/H_2O)

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[(V/V] was used. Acetonitrile for HPLC grade is received from Merck and Water for HPLC grade from Millipore. Various kinds of steroids were used as test substances (Amcinonide, Betamethason-17-valerat, Clobetason-17-butyrat, Clocortolon-21-hexanoat, Clocortolon-21-pivalat, Diflorasondiacetat, Flumetason, Halcinonid, Hydrocortison-17-butyrat and Triamcinolonhexacetonid).

Instrumentation

The column was Lichrospher 100 RP-18 (Merck) with 5 µm diameter and 125 x 4 mm. Water jacket was self constructed. Thermostat was Haake D8 (Fison), Karlsruhe, Germany, with precision ± 0,02 °C. Valve was Rheodyne No. 7010 with a 20 µl loop, Cotati, California. Pump was Bischoff model 2200 (serial 903), Leonberg, Germany. Detector was RI-detector model ERC-7510, Erma Optical Works Ltd. Japan, Range: ¼. Polarity: (-). Recorder: HP-3394, Hewlett-Packard, Waldbronn, Germany. Dead-volume (V_0) was 20 µl. Software: Microsoft Excel and Borland C++.

Procedure

The solvents 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. Dead-time was calculated according to the method of Guardino [7].

RESULT AND DISCUSSION

The retention behavior of n-Alkane homologous series

The retention behavior of n-alkane homologous series is the basic of Kovats Retention Index concept. Therefore, it should be investigated firstly the behavior including some parameters such as: *ln(k') against* n_c, accuracy, *RI against* n_c, *ΔRI against* n_c/n_c+1 and dead time calculation as follows.

ln(k') against n_c

The Retention index system is based on equation (1), i.e. for homologous series of n-alkane exist a linear relationship between ln(k') and carbon number (n_c), (see Fig 1).

$$\ln(k') = a_1 n_c + a_0 \quad (1)$$

Table 1 exhibits a_0, a_1 and r^2, which are derived from Fig 1. The results shown here indicate that r^2 very close to ideal value 1. Slope and intercept increase by the increment of water content, with a

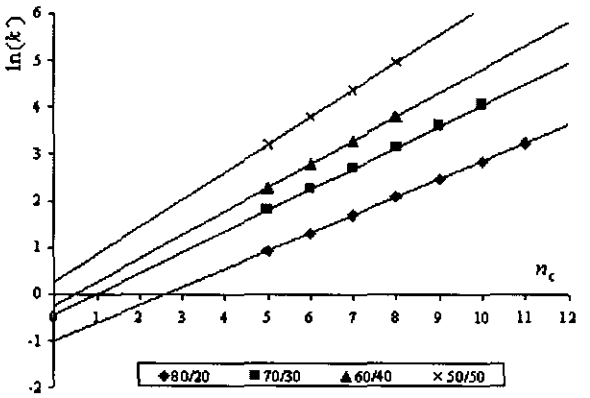

Fig 1. Linear correlation between n-alkane carbon number (n_c) and ln(k') in ACN/H_2O system

Table 1. Regression coefficient values of Fig 1

	$a_0 \pm$ sdv (a_0)	$a_1 \pm$ sdv (a_1)	r^2
80/20	-1.0002 ± 0.0070	0.3850 ± 0.0008	1.0000
70/30	-0.4230 ± 0.0076	0.4476 ± 0.0010	1.0000
60/40	-0.2478 ± 0.0165	0.5052 ± 0.0025	1.0000
50/50	0.2793 ± 0.0711	0.5854 ± 0.0108	1.0000

Table 2. Accuracy of n-alkane retention index in ACN/H_2O system

Substances	n_c	RI/ε	80/20	70/30	60/40	50/50
n-Pentane	5	RI	500.6	499.7	500.2	499.9
		ε	+ 0.6	- 0.3	+ 0.2	- 0.1
n-Hexane	6	RI	599.3	600.5	600.0	600.7
		ε	- 0.7	+ 0.5	0.0	+ 0.7
n-Heptane	7	RI	699.8	699.9	699.8	698.9
		ε	- 0.2	- 0.1	- 0.2	- 1.1
n-Octane	8	RI	800.2	799.7	800.3	800.4
		ε	+ 0.2	- 0.3	+ 0.3	+ 0.4
n-Nonane	9	RI	900.2	899.9	-	-
		ε	+ 0.2	- 0.1	-	-
n-Decane	10	RI	1000.1	1000.1	-	-
		ε	+ 0.1	+ 0.1	-	-
n-Undecane	11	RI	1100.0	-	-	-
		ε	0.0	-	-	-

Table 3. Regression coefficient values and bias of linear relationship between carbon atom number (n_c) and n-alkane retention indices (RI)

ACN/H_2O	b_1	ε (b_1)	b_0	r^2
80/20	100.0046	-0.0046	-0.0053	1.0000
70/30	99.9963	-0.0037	-0.0034	1.0000
60/40	100.0101	-0.0101	-0.0131	1.0000
50/50	99.9943	-0.0057	0.0140	1.0000

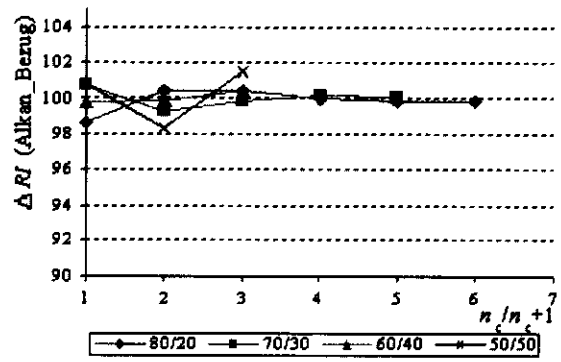

Fig 2. Relationship between ΔRI of n-alkane and n_c/n_c+1 in ACN/H_2O system

Table 4. Dead-time calculation by using of n-alkane homologous series

	80/20	70/30	60/40	50/50
t_0 (min)	1,15	1.00	1,10	1.04
a_1	0.0039	0.0045	0.0050	0.0058
a_0	-0.870	-0.425	-0.145	0.326
n_c	n_5-n_{11}	n_5-n_{10}	n_5-n_8	n_5-n_8
S/N	0.147	0.074	0.033	0.457
RI_5	500.63	499.72	500.17	499.88
RI_6	599.31	600.51	599.97	600.66
RI_7	699.76	699.87	699.81	698.92
RI_8	800.19	799.72	800.26	800.45
RI_9	900.22	899.92	-	-
RI_{10}	1000.13	1000.08	-	-
RI_{11}	1099.99	-	-	-

linear function. Lower n-alkane, e.g. with n_3 or n_4 (Propane or Butane), are in gaseous phase, hence retention index values under 300 till 400 in practice are barely used.

Accuracy

True value of retention index relevance of n-alkane is defined as a fictive number of carbon atom multiplying by 100, e.g. true value for pentane = 500, hexane = 600, heptane = 700, etc. Table 2 shows the accuracy of n-alkane retention index values in ACN/H_2O solvent system.

From all solvent compositions show the bias under 1.0. The tendency shows that the increasing of carbon number can affect better accuracy. The Retention Index was determined according to equation 2.

$$RI = 100\left[\frac{\ln(k') - a_0}{a_1}\right] \quad (2)$$

RI against n_c

Table 3 indicates a linear correlation between retention index (RI) of n-alkane and the Alkane carbon number (n_c) in ACN/H_2O solvent system, whereby the RI-values were determined by using n-alkane as the standard.

The following equation is derived from linear indication:

$$RI = b_1 n_c + b_0 \quad (3)$$

Retention index difference between two neighboring carbon atoms of n-alkane is due to the CH_2 group which is equal 100 [7], as well as the slope (b_1) of equation (3) is defined.

$$\Delta RI_{CH_2} = RI_{n_c+1} - RI_{n_c} = b_1 = 100 \quad (4)$$

ΔRI against n_c/n_c+1

ΔRI serves as the character of retention index difference (eq.4) and n_c/n_c+1 represents the difference of n-alkane carbon atoms respectively, e.g. for Pentane and Hexane is n_c/n_c+1 = 1, for Hexane and Heptane is n_c/n_c+1 = 2, etc. Fig 2 describes this relationship for ACN/H_2O system.

Equation (4) can be used for characterizing of homologous series deviations. In acetonitrile/water system is shown that ΔRI_{CH2} for n-alkane with shorter chain has smaller deviations (only 1–2 units from 100). In acetonitrile/water 80/20 system ΔRI is 99.9 ± 0.6; in 70/30 system ΔRI is 100.1 ± 0.5; in 60/40 system ΔRI is 100.0 ± 0.4 and in system 50/50 is 100.2 ± 1.7. Moreover, it was demonstrated here that deviations for n_c/n_c+1 < 3 were smaller.

Dead-time calculation

The dead-time is calculated by using n-alkane as homologous series in ACN/H_2O system with varying solvent composition as be shown in Table 4. This calculation was adopted from the method of Guardino et al. [6], in which an iteration is carried out upon the dead time (t_0), with slope (a_1) and intercept (a_0) being calculated using the least squares linear regression. The sum of squares of the deviation between calculated value and known Kovats Indices divided by the number of homologous (S of S/N) was used as a general indication of the accuracy of fit [4].

In comparison with different linear methods in GC, the method from Guardino et al. was not only the most accurate, but also the fastest. Similar results have been published by Didaoui et al. [4] by applying this method

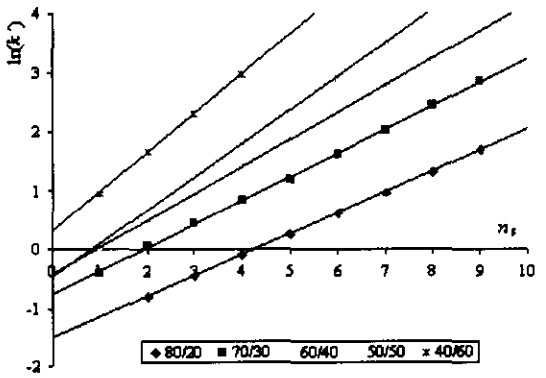

Fig 3. Linear correlation between carbon number of alkyl chain (n_s) and ln(k') in ACN/H_2O system

Table 5. Regression coefficient values (a_1 and a_0) of Fig 3

ACN/H_2O	$a_0 \pm$ sdv (a_0)	$a_1 \pm$ sdv (a_1)	r^2
80/20	-1.5125 ± 0.0151	0.3550 ± 0.0025	0.9999
70/30	-0.7726 ± 0.0439	0.4011 ± 0.0078	0.9995
60/40	-0.4219 ± 0.0399	0.4596 ± 0.0079	0.9997
50/50	-0.4820 ± 0.2091	0.5680 ± 0.0468	0.9949
40/60	0.2962 ± 0.1857	0.6705 ± 0.0678	0.9989

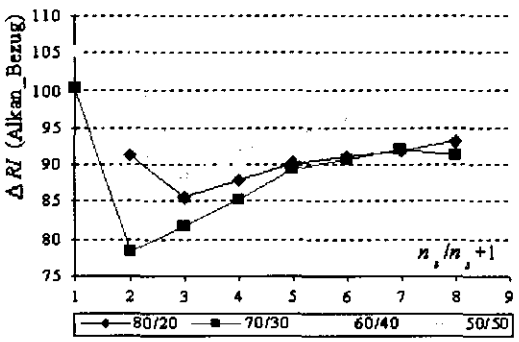

Fig 4. Relationship between ΔRI of alkylarylketone and n_s/n_s+1

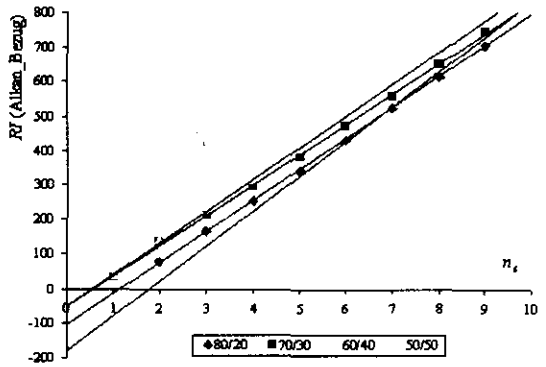

Fig 5. Linear relationship between carbon number of alkyl chain (n_s) and absolute retention indices of alkylarylketone RI (n-alkane scale) in ACN/H_2O system

on the RP-HPLC with various homologous series, such as Alkyl aryl ketones and 1-Nitroalkanes.

Table 4 was the dead-time calculation by using n-alkane homologous series without depending on solvent compositions. In comparison with other homologous series, n-alkane exhibited smaller S of S/N than other homologous series, e.g. Alkan-2-ones, Alkyl benzenes and Alkyl aryl ketones [8].

The retention behavior of alkylarylketone homologous series

Due to very large retention time, then it is suggested using alkylarylketone in connecting with n-alkane as a frame of reference. Some parameters for identifying behavior of alkylarylketone should be investigated such as *ln(k') against* n_s, *ΔRI against* n_s/n_s*+1* and *RI against* n_s then index Kovats can be calculated as absolute retention index as follows.

ln(k') against n_s

The Retention index system is based on equation (5), i.e. for homologous series of alkylarylketone exists a linear relationship between ln(k') and carbon number of alkyl chain (n_s), see Fig 3.

$$\ln(k') = a_1 n_s + a_0 \quad (5)$$

Table 5 exhibits a_0, a_1 and r^2, which are derived from Fig 3. The results shown here indicate that r^2 very close to ideal value 1. Slopes and intercepts increase by the increment of water content and indicate as a linear function.

All r^2-values for both solvent systems are larger than 0.9949. The random error of slope (a_1) and intercept (a_0) was calculated according to Miller et al. [8]. Moreover, the deviations of slopes are smaller than the intercepts. The largest deviations of a_0 and a_1 exhibited in ACN/H_2O system, particularly in composition 40/60.

ΔRI against n_s/n_s*+1*

ΔRI serves as the character of retention index difference and n_s/n_s+1 represents the difference of Alkyl chain carbon number, e.g. for acetophenone and propiophenone is n_s/n_s+1 = 1, for propiophenone and butyrophenone is n_s/n_s+1 = 2, etc. Fig 4 describe this relationship for ACN/H_2O system.

In acetonitrile/water system shown that ΔRI_{CH2} for alkylarylketone with shorter chain (n_s < 5) has a larger deviation. These solvent systems shown that there were great deviations of ΔRI-values between the first two homologous, as well as between the second and the third homologous. Furthermore, ΔRI was 90.2 ± 2.5 in 80/20 system, 87.0 ± 5.3 in 70/30 system, 90.9 ± 3.1 in 60/40 system and 95.0 ± 6.0 for 50/50 system. Generally, it was found that for this solvent system all

Table 6. Regression coefficient values and bias from Fig 5

ACN/H_2O	b_1	$\varepsilon(b_1)$	b_0	r^2
80/20	89.876	10.124	-103.890	0.9999
70/30	87.665	12.335	-49.554	0.9994
60/40	91.921	8.079	-48.374	0.9997
50/50	101.270	1.270	-180.370	0.9913

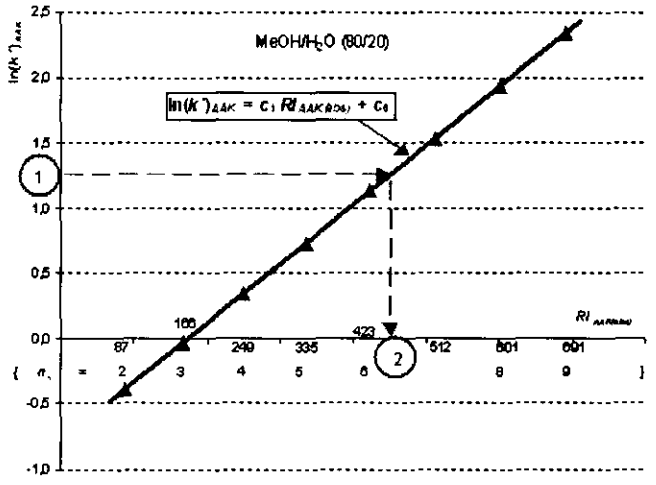

Fig 6. Absolute retention index determination of test substance

ΔRI-values lay fewer than 100.

RI against n_s

A linear correlation between carbon number of alkyl chain (n_s) and Kovats retention indices of alkylarylketone RI (n-alkane scale) in ACN/H_2O system is shown in Table 5. Kovats retention indices of alkylarylketone were calculated by using n-alkane as the standard. Table 6 exhibits b_1, b_0, $\varepsilon(b_1)$ and r^2 derived from Fig 5.

The following expression is obtained from linear equation:

$$RI = b_1 n_s + b_0 \quad (6)$$

$\varepsilon(b_1)$ specify a systematic error of slope b_1, as reference value, where conventionally for this purpose according to Kovats equal to 100 (refer to equation 4). The Kovats retention indices of alkylarylketone in ACN/H_2O system affect a systematic error circa 8, and the average of slopes (b_1) from this linear correlation at this solvent composition (Table 6) is 92. Dead-time calculation (t_0) for Table 6 was calculated by using n-alkane according to iteration procedure [3]. The r^2-values show good correlation between 0.9913 and 0.9999.

Absolute retention index determination

Kovats retention index of substance according to alkylarylketone as the standard can be calculated by connecting with n-alkane as the primary standard. An example here is system MeOH/H_2O (80/20) in Fig 6, where the linear relationship between ln(k') (Fig 3) and Kovats (absolute) retention index values of alkylarylketone (Table 7) are described.

Table 7. Retention Index values of alkylarylketone in ACN/H_2O by using n-alkane as the standard

Substances	80/20	70/30	60/40	50/50
Acetophenone	-	36	38	-115
Propiophenone	77	136	143	42
Butyrophenone	169	215	229	146
Valerophenone	254	297	318	235
Hexanophenone	342	382	408	327
Heptanophenone	433	471	502	421
Octanophenone	524	562	595	517
Nonanophenone	616	654	689	-
Decanophenone	709	745	-	-

Table 8. Kovats (absolute) retention index values of steroids in ACN/H_2O

No	Substances	60/40	50/50
1	Amcinonide	334	320
2	Betamethason-17-valerat	281	261
3	Clobetason-17-butyrat	456	460
4	Clocortolon-21-hexanoat	579	600
5	Clocortolon-21-pivalat	501	512
6	Diflorasondiacetat	231	222
7	Flumetason	361	367
8	Halcinonid	322	306
9	Hydrocortison-17-butyrat	403	394
10	Triamcinolonhexacetonid	534	541

From Fig 6 the following equation could be derived:

$$\ln(k')_{Alkylarylketone} = c_1 RI_{Alkylarylketone(absolute)} + c_0 \quad (7)$$

$\ln(k')_{alkylarylketone}$ is alkylarylketone capacity factor, $RI_{alkylarylketone(absolute)}$ is alkylarylketone Kovats (absolute) retention index and c_1 and c_0 are linear regression coefficients. On the basis of ln(k') against n_s (Fig 3) and RI against n_s (Fig 5) were all linear, consequently, $\ln(k')_{alkylarylketone}$ against retention index absolute ($RI_{alkylarylketone(absolute)}$) must be linear too. Therefore, RI values of Substance based on index system absolute could be determined as follows:

$$RI_{substance} = \frac{\ln(k')_{substance} - c_0}{c_1} \quad (8)$$

$RI_{substance}$ and $\ln(k')_{substance}$ are retention index and capacity factor of substance respectively, whereas c_0 and c_1 are taken from equation (7). The calculation results are shown in Table 8 for ACN/H_2O system, using steroids as substance.

CONCLUSION

n-alkane homologous series (from Pentane) are very suitable as the standard for retention index calculation in the RP-HPLC, although of very non polar and lacking of chromophore properties, and n-alkane fit well in dead time calculation as well. More over n-alkane indicate good reproducibility and is also no representative of this homologous series affected unusual phenomena.

The most important finding in this research is the possibility of adjusting the Kovats retention index system in the HPLC using alkylarylketone homologous series which are then connected with n-alkane as a frame of reference.

ACKNOWLEDGEMENT

The author would like to thank sincerely to DAAD (German Academic Exchange Service) for research stay scholarship and Prof. Dr. S. Ebel for kindly research work supervising.

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