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A retention index system for comprehensive two-dimensional gas chromatography using polyethylene glycols

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Abstract

The characterization and identification of compounds in complex real-world samples is quite difficult and new concepts and workflows are highly desirable. Retention indices (RIs) are widely used in gas chromatography (GC) to support the identification of unknown compounds. Several attempts have been made to introduce a similar concept for the second dimension in comprehensive two-dimensional (2D) GC (GC×GC) but, an easily applicable and robust system remains elusive.

In the present study, a new RI system for GC×GC was developed. Polyethylene glycols (PEGs) were used in combination with a simple linear regression, with *n*-alkanes as reference points for virtually unretained compounds and PEG homologs as reference compounds for second-dimension RIs (PEG-^{2}I). The *n*-alkanes were assigned a PEG-^{2}I of zero and the distance between consecutive PEG homologs from PEG-2 (diethylene glycol) and higher were assigned a PEG-^{2}I value of 10. We used ethylene glycol and PEG-2 through PEG-10 as reference compounds, thereby covering a PEG-^{2}I range from 20.0 for ethylene glycol, over 50.0 for diethylene glycol (PEG-2) to 130.0 for decaethylene glycol (PEG-10); additional PEGs can be added to cover a wider polarity range. The PEG-^{2}I system was initially evaluated using a 30 m × 0.25 mm non-polar (5% phenyl, 0.25 µm film thickness) first-dimension column and a 1.6 m × 0.18 mm polar (50% phenyl, 0.18 µm film thickness) second-dimension column. This system was validated for use with non-polar first-dimension columns and a semi-polar (50% phenyl) second-dimension column, and exhibited robustness to changes in the carrier gas flow velocity, oven temperature ramping rate, and secondary oven temperature offset. An average relative standard deviation of 2.7%, equal to a 95% confidence interval of 1.27 PEG-^{2}I units, was obtained for the PEG-^{2}I values of 72 environmental pollutants. Additionally, the system was found to be applicable over a wide range of boiling points (in the current case, from *n*-heptane to *n*-dotriacontane (C_7-C_{32})) and can be used with various column dimensions. Changing the second-dimension column to either a narrower 0.1 mm column or a wider 0.25 mm column, yielded similar 95%-percentiles to that of the 0.18 mm column, differing by only 3.20 and 2.80 PEG-^{2}I units, respectively. Moreover, methods for improving the system were suggested.

Keywords: GC×GC, retention indices, polyethylene glycols, *n*-alkanes, environmental contaminants

1. Introduction

The possibilities for identifying unknown organic compounds have improved significantly with the introduction of retention indices (RI) in gas chromatography (GC). In 1958, Kováts [1] introduced a relationship between the elution time of a compound and the elution of *n*-alkanes as the basis for RIs associated with isothermal one-dimensional GC. This work has been cited more than 1000 times in the last fifty years [2], and several subsequent studies have proposed other RI calculation methods or modifications to the original relationship. For example van den Dool and Kratz [3] generated alkane or linear retention indices (LRIs) by adapting Kováts' indices for linear oven temperature programming. Similarly Lee *et al.* [4] introduced another RI concept, i.e. the Lee index, which uses polycyclic aromatic hydrocarbons (PAHs) as retention markers (naphthalene, phenanthrene, chrysene, and picene) for the analysis and indexing of polycyclic aromatic compounds (PACs).

The invention of comprehensive 2D GC (GC×GC) by Phillips and Liu in 1991 [5] has yielded considerable improvement in the characterization of complex samples [6]. Most of the early studies employing GC×GC have focused on the analysis of petrochemical samples [7,8]. However, GC×GC is applicable to many other complex sample matrices, e.g., sediment [9], air [10], food extracts [11], and biological samples [12,13]. This technique is particularly well-suited for complex sample characterization and analysis, including non-target analysis [6,14–16] and biomarker identification [17]. Nevertheless, the generation and use of both first-dimension (^{1}D; nomenclature after Schoenmakers *et al.* [18]) and second-dimension (^{2}D) RIs (*I*) is highly desirable.

The development and implementation of a robust RI system for the ^{2}D in GC×GC have, however, proven difficult. In GC, injection of a mixture of *n*-alkanes, or related homologous series of compounds, results in a set of equidistant peaks, because all homologs interact with the GC stationary phase in the same manner. In addition, each CH_2-unit accounts for the same incremental contribution to the vapor pressure of the compounds. Finding RI reference compounds that, in the same manner, produce equidistant peaks in the ^{2}D space have proven challenging. Therefore, iso-volatility curves or iso-volatility plots have been used to generate *I* values similar to the Kováts indices [19–24].

Most methods use continuous or repeated injection of reference compounds, usually *n*-alkanes [19,20,25] or fatty acid methyl esters (FAME) [21], for the creation of "iso-volatility" curves within the 2D separation space. The second retention time (^2t_R) of the injected component decreases exponentially with increasing temperature and the ^2I value is obtained via interpolation (or sometimes extrapolation) between successive alkane lines in the pseudo-isothermal ^{2}D analysis. This method is complex, requires attention to the details of the experiment, valid only for compounds with boiling points within a certain range [19,26], and has poor precision [27]. Several of these drawbacks were overcome via modifications [21,26,28], but application of the method remains complex. Therefore, the ^{2}D space is typically framed by injecting suitable *n*-alkanes or FAMEs, deriving an equivalent ^2I space, and predicting the ^2I values

of solutes found within the calibrated GC×GC space in subsequent sample analyses. However, this approach is vulnerable to (among others) experimental-system variations and matrix effects.

A more theoretical approach, compared with this method, was presented by Dorman *et al.* [24]. They calculated thermodynamic RIs, which were obtained through enthalpy and entropy data and used for predicting the 2D elution of analytes from the Grob mix. Arey *et al.* [29] used stationary phase-gas phase equilibrium partition coefficients to calculate ^{2}D RIs, which were then used to estimate the physico-chemical properties (e.g., aqueous solubility and air-water partition coefficients) needed to predict environmental partitioning. Similarly, Antle *et al.* calculated ^{2}I similar to the Lee RIs and estimated physical properties using the iso-volatility curves of two- to five-ring PAHs to assess the environmental impact on the weathering of coal tar [30].

The present study was aimed at providing a new RI system for the most widely used GC×GC setup, i.e., an apolar×polar column set [31], which may be applied across a wide range of analyte boiling points and with any column dimensions and GC program settings (e.g., carrier gas flow or oven temperature ramping rate). The new system is based on the co-injection of samples and polyethylene glycol (PEG) and *n*-alkane reference standards, which results in both first-dimension LRIs and second-dimension PEG RIs (PEG-^{2}I) for all analytes. The size and polarity of the PEGs increase with increasing number of CH_2CH_2O units and, hence, their chromatographic peaks become distributed along the diagonal of the GC×GC plane, rendering them well-suited for use as retention reference compounds. The repeatability and robustness of the system have been systematically evaluated, pros and cons discussed, and improvements (e.g., expansion of the applicability domain) proposed.

2. Material and methods

The goal of this work was to introduce a new RI system for GC×GC using polyethylene glycols as retention markers. The RIs of 72 compounds were calculated using the work flow presented in section 3.4 and the results for different settings and columns were compared.

2.1. Material

PEGs were purchased from Sigma Aldrich (mono till hexa and octa; Steinheim, Germany) and Tokyo Chemical Industry Co., Ltd. (hepta, nona and deca; Zwijndrecht, Belgium). An aliphatic alcohol mix (C_2-C_8) and an *n*-alkane standard (C_7-C_{40}) were obtained from Chiron AS (Trondheim, Norway) and Sigma Aldrich (Supelco, Bellefonte, PA, USA), respectively. The 8270 MegaMix® standard (hereafter, referred to as MegaMix) was purchased from RESTEK (Bellefonte, PA, USA). This standard consisted of 76 structurally diverse compounds, 72 of which could be analyzed (see **Table 4** in the results section for compound list). Dichloromethane (DCM; 99.99%) was obtained from Fisher Scientific (Loughborough, UK).

2.2. Methods

A mixture consisting of the MegaMix, PEGs, alkanes, and the aliphatic alcohol mix was prepared in DCM. All analyses were performed on an Agilent Technologies 6890 gas chromatograph (Palo Alto, CA, USA) coupled to a Pegasus 4D time-of-flight mass spectrometer (TOF MS; Leco Corp., St. Joseph, MI, USA). A secondary oven and a quad-jet dual stage modulator were built into the main GC oven. The split/splitless injector was operated in splitless mode at a temperature of 280°C. Data were acquired and processed using the Chroma-TOF software (version 4.50; LECO Corp.). The $^{2}t_{R}$ is assigned to each peak, by the software, based on the most abundant sub-peak (i.e., slice). Different GC settings and column configurations were tested. Electron ionization (EI) was performed at an electron energy and an ion source temperature of 70eV and 300°C, respectively. An MS acquisition rate of 100 spectra/s was used for all runs.

2.2.1. Evaluation of the PEG-^{2}I system robustness to variations in the GC settings

Several different GC settings were tested (**Table 1**) to evaluate the robustness of the PEG-^{2}I system. In this set of experiments, a 30-m Rtx-5sil ms (RESTEK) with an internal diameter (ID;$^{1}d_{C}$) and film thickness of 0.25 mm and 0.25 µm, respectively, was used as the first (i.e. primary) column. A 1.6-m BPX-50 with an ID ($^{2}d_{C}$) of 0.18 mm and a film thickness of 0.18 µm (SGE, Trajan Scientific Europe Ltd, Crownhill, Milton Keynes, UK) was used as the secondary column. The temperature program for the first oven started at 35°C and ended at 310°C. Moreover, the modulator had an offset of +20°C relative to the second oven and the transfer line temperature was held at 350°C. The experiments were planned using a design of experiments approach, employing a central composite face-centered design. Three factors were investigated: the temperature ramping rate of the first oven, column flow, and the offset of the secondary oven. A default modulation period of 6 s and hot and cold jet pulses of 0.7 s and 2.3 s, respectively, were applied. In some cases, the modulation period, hot jet pulse, and cold jet pulse were increased to respective values of 9 s, 1.0 s, and 3.5 s (see **Table 1**). The average retention times were used for calculating the RIs.

2.2.2. Evaluation of the PEG-^{2}I system robustness to variations in the GC column dimensions

Three column combinations were used to determine the robustness of the PEG-^{2}I system to changes in the column dimensions (**Table 2**). The default modulation period and hot and cold jet were the same as those stated in section 2.2.1. The third set of experiments, using a narrow-bore second-dimension column, was run with a 4-s modulation (hot jet 0.5 s and cold jet 1.5 s). Furthermore, the temperature program for the first oven started at 35°C and ended at 300°C (first experiment) or 310°C (second and third experiments). The modulator had an offset of +20°C relative to the second oven and the transfer line temperature was held at 330°C and 350°C for the first experiment and the following experiments, respectively.

Table 1 Experimental settings for testing robustness of the proposed new retention index system.

Carrier gas flow (mL/min)	Oven ramping rate (°C/min)	2nd oven offset (°C)	Modulation (sec)	Repetitions
0.8	3	20	9	2
0.8	3	40	6	2
0.8	5	40	6	2
0.8	5	20	6	2
0.8	4	30	6	2
1.25	3	20	6	2
1.25	3	40	6	2
1.25	5	40	6	2
1.25	5	20	6	2
1.25	4	30	6	2
1	4	40	6	2
1	4	20	9	2
1	3	30	9	2
1	5	30	6	2
1	4	30	6	5

3. Theory

3.1. The PEG second dimension retention index concept

The PEG second-dimension RI system is based on the incremental increase in the second-dimension retention associated with one PEG oligomer unit (CH_2CH_2O), which was assigned an index value of 10. Strictly speaking, ethylene glycol lacks the ether function and is therefore not a PEG, but was included in the system to obtain a reference point at short second-dimension retention times; ethylene glycol was assigned an index value based on the PEGs. The reference (zero) point in the PEG-^2I system was defined as the second-dimension retention time (^2t_R) of an *n*-alkane at the LRI of the analyte. The PEG-^2I values are expected (from analyses of alkane and Megamix standards) to range from 0 to ~200, which are significantly lower than the first-dimension LRIs (which are generally ≥500). LRI values can therefore be easily differentiated from their PEG-^2I counterparts. The occurrence of fewer digits also reflects the lower chromatographic resolution and less precise relative retention times in the second dimension (that only span a few seconds), compared with those of the first dimension.

3.2. First-dimension RIs

First-dimension LRIs were generated from *n*-alkanes, as introduced by Kováts (isothermal) and modified by van den Dool and Kratz (temperature programmed) [3]. The LRIs were determined from:

$$LRI = 100 \times \left(n_{\alpha 1} + \frac{t_{R,analyte} - t_{R,\alpha 1}}{t_{R,\alpha 2} - t_{R,\alpha 1}} \right) \quad (1)$$

where $t_{R,analyte}$: retention time of the analyte, $t_{R,\alpha 1}$ and $t_{R,\alpha 2}$: retention times of the *n*-alkane eluting before and after the analyte, respectively, and $n_{\alpha 1}$: number of carbon atoms in *n*-alkane α1.

Table 2 Experimental settings and column configuration for testing robustness of the proposed new retention index system to changes in column dimensions.

1st dimension		2nd dimension		Instrument settings			
$^{1}d_C$ (mm), phase (µm)	Column type	$^{2}d_C$ (mm), phase (µm)	Column type	Carrier gas velocity	Oven ramping	2nd oven offset	Repli-cates
0.25	30m, Rtx-5sil ms	0.25	1.6m, BPX-50	1 mL/min	4°C/min	+30°C	2
0.25	30m, Rtx-5sil ms	0.18	1.6m, BPX-50	1 mL/min	4°C/min	+30°C	5
0.25	30m, Rtx-5sil ms	0.10	1.6m, BPX-50	1 mL/min	4°C/min	+30°C	2

3.3. Second-dimension RIs

The $^{2}t_R$ of "the alkane band" at the LRI of the analyte or PEG marker is taken as the reference (zero) point in our new RI system and, hence, the excess retention ($^{2}t_{R,E}$) had to be interpolated from $^{2}t_R$ values of the *n*-alkanes (**Fig. 1**). This interpolation was performed using a bracketing approach similar to that employed for LRI (Eq. 1). The $^{2}t_R$ values of the alkane band at the $^{1}t_R$ of the analyte are determined as follows:

$$^{2}t_{R,alkane}(analyte) = {}^{2}t_{R,\alpha1} + \frac{{}^{2}t_{R,\alpha2} - {}^{2}t_{R,\alpha1}}{{}^{1}t_{R,\alpha2} - {}^{1}t_{R,\alpha1}} \times \left({}^{1}t_{R,\text{analyte}} - {}^{1}t_{R,\alpha1}\right) \quad (2)$$

where $^{1}t_R$ and $^{2}t_R$ are the retention time in the first and second dimension, respectively. The sub-indices α1 and α2 refer to the alkane eluting before and after the analyte, respectively. Hence, α1 occurs at coordinates ($^{1}t_{R,\alpha1}$; $^{2}t_{R,\alpha1}$), while α2 occurs at ($^{1}t_{R,\alpha2}$; $^{2}t_{R,\alpha2}$). The middle term of the expression (Eq. 2) is equivalent to the slope of the *n*-alkane band in a $^{1}t_R$-$^{2}t_R$-diagram, while the last part refers to the relative positioning ($^{1}t_R$) of the analyte to the alkane eluting before. The $^{2}t_{R,E}$ values (i.e., the distance of the analyte from the alkane band) are obtained by subtracting the $^{2}t_{R,alkane}$ values from the measured $^{2}t_R$ values (**Fig. 1**).

The reference values for the PEG second-dimension RIs (PEG-^{2}I) were obtained via linear regression of the PEG-$^{2}I_{PEGs}$ and $^{2}t_{R,E}$ values of the PEGs. The following simple expression was used to generate suitable PEG reference values:

$$PEG\text{-}{}^{2}I_{PEG}(n) = Offset + 10 \times n_{PE} \quad (3)$$

where n_{PE}: number of polyethylene units and Offset: number of PEG-^{2}I units that had to be added to obtain an intercept of zero. Fitting the data for ethylene glycol to this function, yielded a value of 20. Moreover, a calibration graph was generated from linear regression of the PEG-^{2}I reference values and the corresponding $^{2}t_{R,E}$ values (with zero as the intercept):

$$PEG\text{-}{}^{2}I = slope \times {}^{2}t_{R,E} \quad (4)$$

The PEG-^2I differs conceptually from the LRI. Both indices are generated simultaneously under linear oven-temperature ramping conditions, which is essential for covering a wide range of analyte boiling points and obtaining orthogonality in the GC×GC separation. However, while the resulting LRIs reflect the volatility of the analytes, the PEG-^2I reflect the polarity of the analytes and are essentially independent of the volatility. Each PEG homolog elutes at an incrementally higher temperature and is separated under pseudo-isothermal conditions. Thus, their ^{2}D retention depends solely on specific analyte-stationary phase interactions. The polarity of the PEG and the ^2t_R values increase with increasing number of ether units in the PEG chain.

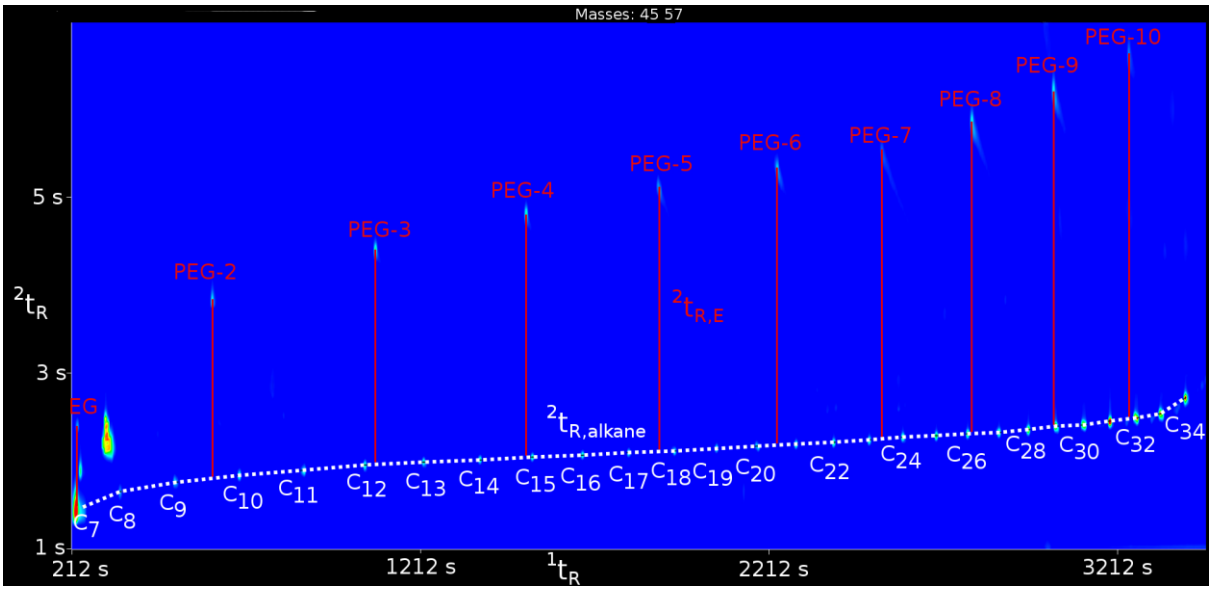

Fig. 1. Chromatogram showing n-alkane (C_7 – C_{34}) and PEG elution (PEG-2 – PEG-10 and EG). $^2t_{R,E}$ and $^2t_{R,alkane}$ are indicated by vertical solid lines (red) and horizontal dashed lines, respectively.

In rare cases, analytes may interact less with the stationary phase than the *n*-alkanes. Methyl siloxanes exhibit, for example, almost zero activity on siloxane-based stationary phases. In such cases, the PEG-^2I, although negative, can still be calculated.

3.4. Application of PEG-^2I second dimension retention indices

The RI system can be successfully applied in three steps, which are given as follows: (i) use Eq. (2) to determine the $^2t_{R,alkane}$ values corresponding to the analytes, (ii) calculate the $^2t_{R,E}$ of each analyte, and (iii) use Eq. (4) to determine the PEG-^2I of each analyte.

The PEG-^2I of azobenzene, as an example, is calculated as follows (the data required for the calculation can be found in **Table S3)**.

i. The *n*-alkanes hexadecane and heptadecane are eluting before and after azobenzene, respectively. Using the numbers from **Table S3** and Eq. (2) from above, yields the following equation:

$$^{2}t_{R,alkane}(azobenzene) = 2.07 + \frac{2.105 - 2.07}{1808 - 1676} \times (1712 - 1676) = 2.08$$

ii. The distance of azobenzene from the alkane band is determined from:

$$^{2}t_{R,E} = {}^{2}t_{R,azobenzene} - {}^{2}t_{R,alkane}(azobenzene)$$

$$^{2}t_{R,E} = 4.53 - 2.08 = 2.45$$

The $^{2}t_{R,E}$ values of all PEGs are calculated in this manner (**Table S3**) and a linear function, PEG-^{2}I (**Table 3**) vs. $^{2}t_{R,E}$ (with a slope of 29.33), is obtained (Eq. 3).

iii. The PEG-^{2}I of azobenzene is then obtained from:

$$PEG\text{-}^{2}I\ (azobenzene) = 29.33 \times 2.45 = 71.9$$

Table 3 PEG-^{2}I for the retention index markers used in this study

Compound	Molecular formula	Abbreviation	PEG-^{2}I
n-alkanes	C_nH_{2n+2}	C_n	0
Ethylene glycol	$C_2H_6O_2$	EG	20
Diethylene glycol	$C_4H_{10}O_3$	PEG-2	50
Triethylene glycol	$C_6H_{14}O_4$	PEG-3	60
Tetraethylene glycol	$C_8H_{18}O_5$	PEG-4	70
Pentaethylene glycol	$C_{10}H_{22}O_6$	PEG-5	80
Hexaethylene glycol	$C_{12}H_{26}O_7$	PEG-6	90
Heptaethylene glycol	$C_{14}H_{30}O_8$	PEG-7	100
Octaethylene glycol	$C_{16}H_{34}O_9$	PEG-8	110
Nonaethylene glycol	$C_{18}H_{38}O_{10}$	PEG-9	120
Decaethylene glycol	$C_{20}H_{42}O_{11}$	PEG-10	130

4. Results

4.1. Characteristics of the PEG RI model

The size and polarity of the PEGs increase with increasing number of PEG oligomer (CH_2CH_2O) units and, hence, their chromatographic peaks are distributed along the diagonal of the GC×GC plane, equidistant in both first and second dimension, rendering them well-suited for use as retention reference compounds. In practice, generating a true orthogonal system, by uncoupling the separation in both dimensions, is only partly accomplished using GC systems. Consequently, many analytes generally appear along the diagonal of the GC×GC plane.

In the proposed second-dimension RI system, the *n*-alkanes have (by definition) a PEG-^{2}I of zero. The PEG-^{2}I of the PEG reference compounds used in this study (see **Table 3**) were obtained from Eqs. (3) and (4) in section 3.3. The PEG reference values were correlated with the corresponding $^{2}t_{R,E}$ values, yielding the calibration graph shown in **Fig. 2**.

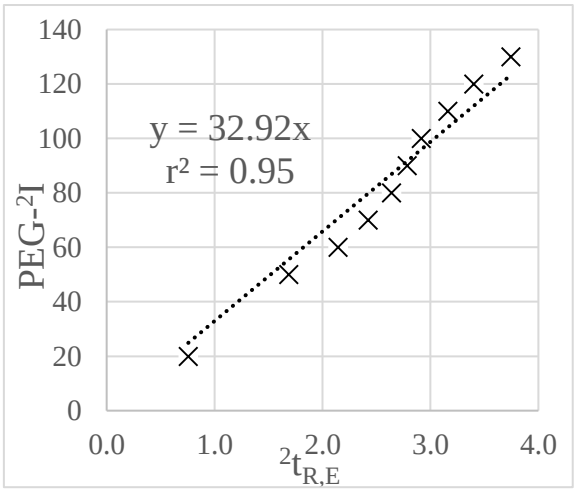

Fig. 2. Calibration graph for determining the PEG-^{2}I from the corrected $^2t_{R,E}$ values associated with a 4°C/min oven temperature heating ramp, a 1 mL/min flow rate, and a 30°C offset of the second oven relative to the first oven.

The PEG reference compounds cover a wide range of boiling points, from that of *n*-heptane (C_7) to those of *n*-dotriacontane (C_{32}) and most of the analytes in the MegaMix standard. Only PAHs with five or six fused rings elute significantly after PEG-10 (with PEG-^2I values of 150–210).

4.2. Repeatability

The center point of the central composite face-centered design was repeated five times, as shown in **Table 1**. The maximum relative standard deviation (1.8%) of all the compounds and an average of 0.70% are indicative of very reliable data acquisition.

4.3. Robustness

4.3.1.Evaluation of the PEG-^2I system robustness to variations in the GC settings

Table 4 shows the average RI calculated for 72 compounds and 14 different experiments performed in duplicates (see **Table S1** and **S2** in the supplementary information for all PEG-^2I and LRI values). An average relative standard deviation (RSD) of 2.7% was obtained with a maximum of 7.7% for benzo(g,h,i)perylene. However, large PAHs eluted after PEG-10 in both the first and second dimension and, hence, the corresponding RI calculations had to be extrapolated. Neglecting the extrapolated compounds yielded a slightly better, i.e., lower (2.3% vs. 2.7%), average RSD and a lower maximum (4.6% for phenol vs. 7.7% for benzo(g,h,i)perylene). The 95% confidence interval was calculated for each analyte across the 14 runs, and the average confidence interval (1.3 PEG-^2I units) decreased to 0.8 PEG-^2I units when extrapolated compounds were excluded. In addition, the method was tested using a constant pressure as well as a ramped pressure, both with an average flow rate of 1mL/min, and comparable results were obtained.

Table 4 Analytes and their corresponding retention index (LRI and PEG-^2I; average over 14 runs with different conditions) sorted in order of first dimension retention time.

Analyte	LRI	PEG-^2I	PEG-^2I RSD	Analyte	LRI	PEG-^2I	PEG-^2I RSD
Aniline	974	62.5	4%	1,2-Dinitrobenzene	1457	115.5	1%
Phenol	978	43.5	5%	3-Nitroaniline	1480	116.0	1%
Bis(2-chloroethyl) ether	984	42.5	4%	Acenaphthene	1483	78.0	2%
2-Chlorophenol	987	42.8	3%	Dibenzofuran	1516	75.1	2%
1,3-Dichlorobenzene	1003	35.5	3%	Dinitrotoluene	1525	85.0	1%
1,4-Dichlorobenzene	1013	37.1	3%	4-Nitrophenol	1525	89.3	2%
1,2-Dichlorobenzene	1030	42.9	2%	2,3,5,6-Tetrachlorophenol	1538	65.8	2%
2-Methylphenol	1033	55.7	3%	2,3,4,6-Tetrachlorophenol	1546	67.4	2%
Bis(2-chloroisopropyl) ether	1052	28.0	4%	Fluorene	1583	78.7	2%
4-Methylphenol	1052	46.0	3%	Diethyl Phthalate	1585	68.5	2%
N-Nitrosodi-N-propylamine	1068	40.2	3%	4-Chlorodiphenylether	1593	65.6	2%
Hexachloroethane	1073	32.6	3%	4-Nitroaniline	1597	138.3	1%
3-Methylphenol	1073	45.9	3%	Dinitro-o-cresol	1602	74.4	1%
Nitrobenzene	1084	65.4	2%	Diphenylamine	1619	85.8	1%
Isophorone	1119	43.7	1%	Azobenzene	1624	72.8	2%
2-Nitrophenol	1129	58.8	2%	4-Bromodiphenylether	1692	74.7	2%
2,4-Dimethylphenol	1146	45.8	2%	Hexachlorobenzene	1693	63.8	4%
Bis(2-chloroethoxy)methane	1158	47.6	3%	Pentachlorophenol	1743	77.2	3%
2,4-Dichlorophenol	1166	50.2	2%	Phenanthrene	1780	100.8	3%
1,2,4-Trichlorobenzene	1175	44.9	2%	Anthracene	1791	98.6	3%
Naphthalene	1184	62.0	1%	Carbazole	1839	123.2	2%
p-Chloroaniline	1199	78.8	2%	Dibutyl phthalate	1951	56.9	1%
Hexachloro-1,3-butadiene	1210	29.0	3%	Fluoranthene	2061	116.1	4%
4-Chloro-3-methylphenol	1287	55.8	2%	Pyrene	2115	129.1	4%
2-Methylnaphthalene	1295	57.9	2%	Benzyl butyl phthalate	2338	92.7	2%
1-Methylnaphthalene	1310	63.7	2%	Bis(2-ethylhexyl) adipate	2386	19.6	2%
Hexachlorocyclopentadiene	1326	34.8	3%	Benz[a]anthracene*	2451	136.4	5%
2,4,6-Trichlorophenol	1349	55.2	2%	Chrysene*	2462	142.9	5%
2,4,5-Trichlorophenol	1354	56.2	3%	Bis(2-ethylhexyl)phthalate	2527	40.5	2%
2-Chloronaphthalene	1376	66.7	2%	Di-n-octyl phthalate	2722	44.6	3%
2-Nitroaniline	1401	105.4	1%	Benzo[b]fluoranthene*	2766	155.1	5%
1,4-Dinitrobenzene	1429	99.9	1%	Benzo[k]fluoranthene*	2774	155.7	5%
1,3-Dinitrobenzene	1442	97.0	1%	Benzo[a]pyrene*	2856	176.5	6%
Dimethyl phthalate	1446	79.8	2%	Indeno[1,2,3-cd]pyrene*	3172	191.7	7%
Acenaphthylene	1449	83.3	2%	Dibenz[a,h]anthracene*	3185	186.0	6%
2,6-Dinitrotoluene	1451	93.2	2%	Benzo[ghi]perylene*	3241	212.1	8%

**: PEG-^2I was extrapolated*

4.3.2.Evaluation of the PEG-^2I system robustness to variations in the GC column dimensions

As previously mentioned, PEGs, alkanes, and the MegaMix compounds were analyzed using different columns. **Table 5** shows the deviation of the average PEG-^2I, from the results in section 4.3.1. The maximum deviation for the 0.1 mm ID column was obtained for benzo(g,h,i)perylene, the last eluting compound in both the first and second dimension. The maximum among the interpolated data for the 0.1 mm ^2d_C was obtained for phenol.

5. Discussion

5.1. Robustness of PEG-^2I values to changes in the GC settings

The calculated RIs were stable to changes in the GC×GC parameters, such as the flow rate, the GC oven ramping rate, and the temperature bias between the first- and second-column ovens. Compared with the variation associated with other compounds, the variation was larger for early and late-eluting compounds (**Fig. 3**). Such effects frequently occur in GC×GC when the oven temperature program contains

isothermal segments (e.g., [30]). Early eluting compounds are affected by the injection process, solvent and stationary phase refocusing effects, and the initial isothermal period. The effect of starting temperature on early eluting compounds have been previously investigated [32]. Late-eluting compounds are affected by the isothermal period at the end of each run and by contributions from the column segment in the transfer line to the overall retention. In general, the non-linearity problem seems to be more severe for late-eluting compounds than for early eluting compounds. However, a large region in the middle of each chromatogram exhibits linear behavior. Good repeatability and robustness were obtained for compounds within the method domain (interpolated compounds) with an average 95% confidence interval of 0.76 PEG-^{2}I units.

Table 5 Deviation of measured PEG-^{2}I from PEG-^{2}I obtained with different GC settings. Relative deviation given in % and percentile in PEG-^{2}I units.

Analyte type		$^{2}d_C$ 0.1mm	$^{2}d_C$ 0.18mm	$^{2}d_C$ 0.25mm
Interpolated and extrapolated compounds	Minimum	0.04%	0.00%	0.03%
	Maximum	22.5%	1.9%	3.4%
	Average	2.7%	0.7%	1.7%
	95%-percentile	8.9 units	1.3 units	3.5 units
Only interpolated compounds	Minimum	0.04%	0.00%	0.03%
	Maximum	7.2%	1.9%	3.4%
	Average	1.9%	0.7%	1.7%
	95%-percentile	3.20 units	1.23 units	2.80 units

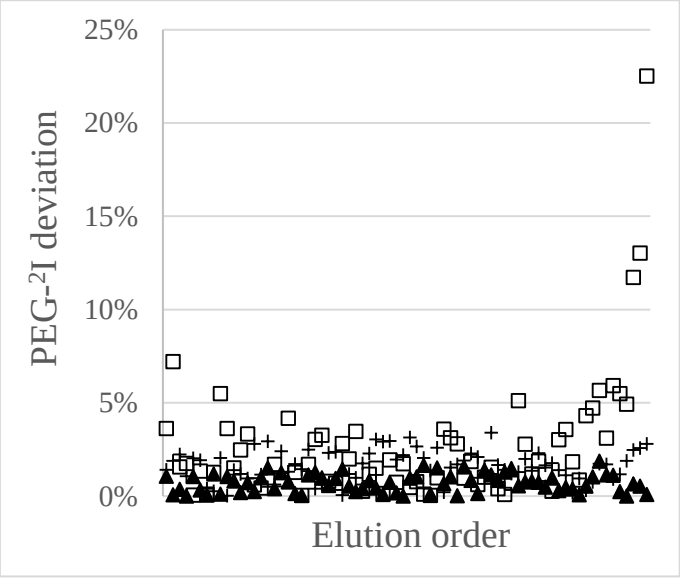

Fig. 3. PEG-^{2}I deviation from the average for each compound using a 0.10 mm $^{2}d_C$ column (open squares), 0.18 mm $^{2}d_C$ column (filled triangles), and 0.25 mm $^{2}d_C$ column (+).

At very short (below LRI 1000) or very long (above LRI 3200) first-dimension retention times, the alkane band exhibits non-linear behavior (**Fig. 1**). This trend was also observed in the PEG-^{2}I calibration plots (**Fig. 2**). Attempts to correct this behavior yielded only slight improvements, but additional methods for improvement have been considered (see section 5.3).

5.2. Robustness of PEG-^2I values to changes in the GC-column configuration

The robustness of the system using a 0.18 mm ^2d_C was demonstrated and, hence, further experiments were conducted to determine the influence of changes in the secondary column diameter on this robustness. To determine the absolute variation among the experiments, the 95%-percentiles were calculated for three column combinations using a conventional 5%-phenyl first-dimension column (0.25 mm ID and 0.25 µm film thickness) and 50%-phenyl ^2d_C with the same phase ratio as the first column, but varying IDs. Similar results were obtained for the 0.18 mm and 0.25 mm ^2d_C, but the narrower 0.10 mm ^2d_C second-dimension column yielded lower PEG-^2I precision for both interpolated and extrapolated compounds (**Table 5**). Acceptable variation (3.20 PEG-^2I units) was obtained for the interpolated compounds, but extremely high (>5 PEG-^2I units), i.e., unacceptable, values were obtained for extrapolated compounds, such as indeno(1,2,3-c,d)pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene. This may have resulted from the elution of those analytes during the isothermal part of the temperature program. However, owing to dispersion, the uncertainty among late-eluting compounds is larger (in general) than that associated with early eluting compounds and, hence, determination of the peak apex is more difficult. The relatively poor precision (7.2% RSD) obtained for the interpolated compound phenol, is attributed to its early elution (PEG-^2I 43.5). For early eluting compounds, even a small variation will yield a substantial RSD.

The generally larger variation in the results obtained for 0.10 mm ^2d_C (**Fig. 3**), compared with the variation occurring at 0.18 mm and 0.25 mm ^2d_C, is most likely attributed to several factors. For example, the pressure calculations are rendered extremely complex by the large difference in the IDs associated with ^1d_C (0.25 mm in all experiments) and ^2d_C. We used the constant flow mode (set at 1 mL/min) and, hence, throughout the run, the GC×GC software continuously calculates the head pressure required to generate this flow. Although the software is expected to handle the conventional column dimensions and GC parameters reasonably well, viscosity effects in the transfer line may prove challenging. Furthermore, the viscosity of the mobile phase increases with the temperature and the transfer line is kept at a constant high temperature, leading to an additional backpressure. This backpressure will increase with decreasing ^2d_C diameter and, thus, will be greatest for the narrowest column. The pressure increase may result in both systematic variations (e.g., stemming from the inlet head pressure calculations) and random variations (e.g., stemming from the temperature regulation of the transfer line) that could account for the larger variation in the more narrow column. However, other factors may be of importance as well.

5.3. Further improvements

5.3.1.Improving the linearity of the PEG-^2I calibration function

If volatile analytes are of interest, then split-injection combined with a linear GC oven temperature program without an initial isothermal period may improve the precision of the PEG-^2I values. Similarly, for high-boiling compounds the linearity could be improved at the end of the analysis by using a less

retentive first-dimension column, such as a shorter column or a column with a thinner film than that used in the current measurements. The use of a deactivated uncoated capillary in the transfer line may also further improve the linearity. Furthermore, the use of a column combination with small or no differences in the $^{1}d_C$ and $^{2}d_C$ internal diameters is recommended, for reasons discussed above.

5.3.2.Substituting EG when analyzing semi-volatile analytes

The solvent used in this study, DCM, has a rather low boiling point (~40°C) that allows the use of a short solvent delay and analysis of early eluting compounds. However, when less volatile analytes are evaluated, solvents with boiling points higher than 40°C (e.g., toluene: boiling point of ~111°C) are often used, and the solvent delay must be increased. Detection of the first early eluting marker compounds (e.g., EG, which has an LRI of 699) would then be impossible. In such cases, we recommend the use of 1-octanol (LRI: 1070, PEG-^{2}I: 17) as an early eluting retention marker.

5.3.3. Increasing the PEG range

Several late-eluting compounds, mainly large PAHs, had to be extrapolated. Larger variations occurred for these compounds compared with those obtained for the interpolated compounds. Therefore, an increase in the range of analyzed PEGs would improve the determination of PEG-^{2}I for compounds eluting late in the second dimension. However, caution is required in these cases to ensure that the compounds elute before the final isothermal in the end of the run. This (as previously mentioned) may be achieved by using a less retentive first-dimension column, than that used in the current study.

5.3.4.Using alternative retention markers to PEGs

PEGs are rather polar and typically tail, especially as the column ages. Therefore, the use of methylated PEGs (glymes), which exhibit a lower polarity and, hence, less tailing than PEGs, may be preferred in some cases. This may yield some improvement for samples containing non-polar or moderately polar/polarizable compounds, such as non-aromatic petroleum fraction and many flavor and fragrance samples. **Table 6** shows the PEG-^{2}I s of several glymes.

Table 6 PEG-^{2}I and LRI values for glymes as alternative markers

Compound	**Molecular formula**	**LRI**	**PEG-^{2}I**
Diglyme	$C_6H_{14}O_3$	943	26.8
Triglyme	$C_8H_{18}O_4$	1227	37.0
Tetraglyme	$C_{10}H_{22}O_5$	1499	44.1
Pentaglyme	$C_{12}H_{26}O_6$	1770	50.9
Hexaglyme	$C_{14}H_{30}O_7$	2133	57.3
Heptaglyme	$C_{16}H_{34}O_8$	2313	62.2
Octaglyme	$C_{18}H_{38}O_9$	2576	69.1
Nonaglyme	$C_{20}H_{42}O_{10}$	2837	77.1
Decaglyme	$C_{22}H_{46}O_{11}$	3107	87.7

If glymes are used in place of PEGs, the second and third step of the procedure described in section 3.4. must be adapted by establishing a linear regression model for the PEG-^{2}I and $^{2}t_{R,E}$ values of the glymes. After the slope of the line is calculated, PEG-^{2}I values can be obtained directly from the $^{2}t_{R,E}$ values.

5.3.5.Polyethylene glycols as analytes

For the analysis of PEGs, deuterated PEGs can be added to samples as retention-time reference points, linking samples to a calibration function developed using a separate run with PEGs and their deuterated analogs. This would provide the necessary information for calculating PEG-^{2}I values, and allow determination of PEG targets.

6. Conclusions

A method is presented for establishing and applying a new RI for the second dimension retention in GC×GC (PEG-^{2}I), using a non-polar first-dimension column and a semi-polar (50% phenyl) second-dimension column. This method exhibited excellent repeatability and robustness to changes in the GC settings, column dimensions, and stationary-film thickness. Sample constituents can all be characterized by three identifiers (an LRI, a PEG-^{2}I value, and a mass spectrum) and, hence, this new system will enhance our ability to thoroughly characterize or identify compounds, via GC×GC-MS, in complex mixtures. In many cases, highly organized patterns of compounds ("group-type patterns") are formed on the 2D plane, with compounds sharing a certain functional group (e.g. alkanes, fatty acid methyl esters) lining up in a logical and structured manner. Such patterns may provide additional support to the identification process.

An Excel sheet with an example can be provided upon request.

7. References

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