ELSEVIER

Journal of Chromatography A, 724 (1996) 229–234

JOURNAL OF CHROMATOGRAPHY A

Simple calibrated merging of gas chromatography capillary column temperature-programmed retention-index compilations

R. Richmond

Structure Elucidation Group, Preclinical Research department, Sandoz Pharma AG, CH-4002 Basel, Switzerland

Received 11 May 1995; revised 31 July 1995; accepted 22 August 1995

Abstract

Experimental calibration methods were used to generate gas chromatographic retention index offset plots to allow the comparison of separate temperature-programmed retention index datasets, for the eventual purpose of merging them. The 8 °C/min on an OV-1 stationary phase subset of the Sadtler Standard Gas Chromatography Retention Index Library was used as an initial model. After merging, individual experimental search windows were assigned to each of the structures from the incorporated datasets.

Keywords: Retention indices; Temperature-programmed retention indices

1. Introduction

Capillary column GC can generate intrinsically very precise retention indices (RI), assuming standardisation of stationary film polarity, carrier gas flow-rate, stationary phase film thickness and temperature-programming rate. This could have great promise for the identification of unknown eluates. However since the majority of the published RI literature has not been produced under conditions in which these four parameters were strictly comparable, much of this intrinsic precision in retention indices and thus the comparability and utility for databases is, for practical purposes, lost.

Several large RI datasets have been published (Sadtler Laboratories [2], Jennings and Shibamoto [3] and Pfleger et al. [9]). An initial idea to merge the datasets was by adopting one dataset as a founding kernel (i.e. the 8 °C/min on the OV-1 stationary phase subset of the excellent Sadtler compilation), then establishing experimental conditions that mimic this kernel and integrating other databases into the resultant 'pseudo-Sadtler' database, using a plot of empirically determined RI offsets for each dataset. Temperature-programming retention indices (TPRI) rather than isothermal indices were chosen to extend the range of structures able to be included. The 8 °C/min rather than the 2 °C/min Sadtler dataset was chosen as the target to model as it was closer to the fast temperature-programming rates found in forensic toxicology, where there are several small datasets of polar structures with the potential for incorporation.

Calibration (offset) plots were created by taking a representative and diverse structure subset of the dataset to be incorporated and measuring the RI under the established 'pseudo-Sadtler' conditions to give RI_{exp} values. The differences between the published index (e.g. RI_{Sad}, RI_{Jenn} and RI_{Pfl}) and the RI_{exp} value of the selected structures were then

0021-9673/96/$15.00
SSDI 0021-9673(95)00924-8

plotted against the appropriate RI range. A critical factor is the need to measure enough structures to accurately represent the scatter of the RI offsets.

Therefore the objectives of this study were firstly to try and find gas-chromatography/mass-spectrometry (GC–MS) conditions using helium carrier gas that could very accurately emulate the GC conditions of the Sadtler database, which used hydrogen carrier gas. The GC–MS can screen out column bleed at high column temperatures, increasing sensitivity and therefore decreasing loading dependent peak asymmetry, and so improving RI reproducibility, compared with normal GC. After the creation of a RI offset plot using a representative subset of structures contained within the Sadtler dataset, the entire Sadtler dataset could be merged into the 'pseudo-Sadtler' database. Individual search windows can be assigned from the 0.95 prediction limits derived from the polynomial regression fit to the offsets. A second goal of this study, was to extend this methodology to the Jennings and Shibamoto [3] and Pfleger et al. RI collections [9] and then compare the assigned search windows with those for the largest already published RI compilation, e.g. the TIAFT collection [8] with its recommended search window of ±50–60 RI units.

Some comments on the three datasets that were evaluated are warranted. The Sadtler 8 °C/min on OV-1 dataset has 1872 entries with useable RI_{Sad} values (extending from 600 to 2538), but has a relative paucity of complex polar structures. Typical structures are polyaromatics, phthalate esters, alkyl amines, phosphate esters, fatty acid esters, alkyl benzenes, alkyl and benzyl ketones, alkyl alcohols, chloroalkanes and chlorobenzenes, bromoalkanes and bromobenzenes. Consequently, the 357 organic molecules measured under the 'pseudo-Sadtler' conditions were heavily supplemented by polar drug structures with RI_{exp} values both below and above 2583 to try and address this problem e.g. metharbital, mephenytoin, trazadone, flunarizine and buspirone. Of the 357 structures, 323 were in the RI_{exp} range 600 to 2583 (RI_{Sad} limits), 9 in the RI_{exp} range 2583 to 2800 (the temperature programming ends at 2800) and 25 in the RI_{exp} range 2800 to 3593. As for the Jennings and Shibamoto 'flavours' dataset [3], after elimination of ambiguous and duplicated structures there are 1186 entries with useable RI_{Jenn} (OV-101) values, in the range 350 to 2155. Representative structures in this dataset include fatty acid esters, alkyl and benzyl ketones, alkyl alcohols, alkyl benzenes, alkyl thiophenes, alkyl furans, alkyl thiazoles, alkyl pyrazines, alkyl thiazoles, monoterpenes and sesquiterpenes. The Pfleger dataset [9] has 3370 entries with useable RI_{Pfl} (OV-101) values ranging from 1005 to 3910 and typical structures include amphetamines, diphenol laxatives, barbiturates, benzodiazepines, butyrophenone and bis-fluorophenyl neuroleptics, phenthiazine, alkanolamine, alkylamine, ethylenediamine and piperazine antihistamines and opioid analgesics.

2. Experimental

2.1. Calibration

The base/neutrals mixture for EPA method 625 (Sigma part number 38,463-1; 41 structures in total), was manually injected in a purged splitless mode, simultaneously with a C_5 to C_{28} *n*-alkane mixture (Sadtler Marker Kit No. 40, mixture no. 6) and the retention indices measured by simple linear interpolation between bracketing *n*-alkanes, using the Van den Dool and Kratz equation [6]. This was used firstly to find the 'pseudo-Sadtler' conditions and later in the study acted as a daily control. Of the 41 structures, 30 are in the Sadtler data set, although one of these 30 (i.e. 4-chlorophenyl phenyl ether) was discarded as an obvious mistake in the Sadtler database. Constant pressure rather than constant flow conditions were used as recommended by Sun et al. [1]. A least squares unweighted fit of these 29 retention indices with the RI_{Sad} values allowed adjustment of the temperature programming (13 °C to 18 °C/min) and helium flow-rate (50.0 to 80.0 kPa head pressure) to find the best alignment i.e. the pseudo-Sadtler conditions. These were a linear temperature-programming rate of 17 °C/min from 35 to 300°C and a HP 5890A GC column head-pressure of 62.5 kPa helium (=1.5 ml/min at 20°C). These two values were carefully chosen to minimize the various hidden influences causing irreproducibility in the system, e.g. column film thinning and low resolution of the gas flow-rate parameter. In general, the programming rate was considered an easier parameter to manipulate and so was used for final fine tuning rather than the gas flow-rate due to the slight

irreproducibility of the GC pressure gauge settings [7]. The 17 °C/min rate has the fortuitous consequence of bridging the gap between the gas chromatographic theoreticians who use slow programming rates to minimize temperature gradients across stationary phase films, and the forensic toxicologists who use relatively fast programming rates.

2.2. Instrumentation

The stationary phase OV-1 (Hewlett-Packard Ultra-1) was chosen to maximize the molecular weight range in eluted species. All 357 structures were measured with simultaneous injection of *n*-alkane standards (C_5 to C_{40} inclusive except C_{39} which was not commercially available). A Hewlett-Packard Ultra-1 capillary column (25 m ×0.32 mm I.D. and a film thickness of 0.52 μm) was used for consistency with the Sadtler compilations. The columns were monitored for performance deterioration by observing the relative height and asymmetry of the relatively polar 3,3′-dichlorobenzidene peak (the structure most susceptible to column deterioration) relative to the adjacent apolar chrysene peak in the base/neutrals calibration mixture for Environmental Protection Agency (EPA) Method 626. If a loss in either relative peak height or symmetry for 3,3′-dichlorobenzidene was seen the column was immediately washed with 10 ml of dimethylsulphoxide and then 10 ml of dichloromethane using a capillary column washing reservoir (Scientific Glass Engineering, Milton Keynes, UK). In all cases, the response and symmmetry of the 3,3′-dichlorobenzidene peak returned, allowing the entire study to be conducted using one (six times washed) HP Ultra-1 column.

A Finnigan TSQ-70B quadrupole mass spectrometer was used as a GC detector, running in 70 eV electron-impact ionisation mode, coupled with a Hewlett-Packard 5890 Series II gas chromatograph. The GC injector was maintained at 250°C, the GC transfer line at 300°C, the source at 150°C and the vacuum manifold at 70°C. The mass range was scanned from 10 to 770 m/z in 0.5 s. The GC program was from 35°C to 300°C at 17 °C/min, followed by 30 min isothermally at 300°C. Carbagas helium grade '60' was used after being passed over a drying bed. The mass spectrometer electron multiplier was at +1000 V, the conversion dynode at −15 kV and the electron current at 400 μA.

Linear and polynomial fits (with prediction limits) were made using the non-linear least squares regression facility of the technical graphics program ORIGIN (Microcal Software, USA).

3. Results and discussion

The initial optimisation experiments to model the Sadtler 8 °C/min on the OV-1 dataset proved successful. Table 1 illustrates the sum of the residual squares, of unweighted linear regression fits between the RI_{Sad} values and the retention indices for the 29 structures from the base/neutrals mixture under various temperature-programming and helium carrier flow conditions. The sum of the residual squares proved more informative than the correlation coefficient when chosing from a series of very good linear fits. The combination of 62.5 kPa helium head pressure and a 17 °C/min programming rate was chosen because of both its relatively low sum of the

Table 1
The sums of the residual squares (i.e. a measure of the scatter of the *y* data about the regression line Ref. [13]) from unweighted linear regression fits

Temperature	50.0 kPa	60.0 kPa	62.5 kPa	65.0 kPa	67.5 kPa	70.0 kPa	80.0 kPa
13 °C/min	84.89	108.44	146.60	124.92	165.14	169.05	243.21
14 °C/min	57.41	82.66	91.89	92.35	99.31	103.33	167.95
15 °C/min	49.86	53.21	88.54	61.42	70.34	68.02	99.96
16 °C/min	65.94	56.96	52.71	52.23	54.00	76.67	78.83
17 °C/min	86.10	58.64	54.42	52.94	54.30	53.81	86.53
18 °C/min	99.56	64.74	59.84	70.06	69.23	61.82	65.44

The sum of the residual squares=degrees of freedom×$(STEYX)^2$, where STEYX is the Microsoft Excel spreadsheet function for the standard error of the *y* estimate, with degrees of freedom=27. The *x* data points are the RI_{Sad} values (8 °C/min on OV-1) for the 29 structures in the base/neutrals mixture, and the *y* data points are their respective experimental retention indices, when the helium head pressure and GC temperature-programming rate were varied.

residual squares and its distance from any sudden jump in this parameter. In total 357 diverse structures were run under the 'pseudo-Sadtler' conditions. Of these, 137 had RI_{Sad} values and 94 had RI_{Jenn} values. Care was exercised in the choice of these pivotal 'overlapping' structures to maximize the uniformity of the spread across the entire TPRI range.

As for the precision of the data, Fig. 1 shows the standard deviation of the experimental entries with $n=6$ for each point. Two outliers were excluded due to obvious chromatographic tailing, giving a total of 355 points. This data was fitted unweighted with a second order polynomial to show firstly that there is a dependence of the standard deviation on the RI_{exp} and secondly that even at $RI_{exp}=2800$ the predicted standard deviation is only equal to 1.34. Three reasons for this dependence could be firstly that the GC temperature programming ends above $RI_{exp}=2800$, resulting in peak broadening, secondly that the majority of structures with $RI_{exp}>2800$ are highly polar drugs which are more susceptible to active site adsorption effects and thirdly that the performance of the Van den Dool and Kratz equation deteriorates at high elution temperatures [12].

When a simple unweighted linear regression of the RI_{exp} (134 points rather than 137 due to the outlier elimination) versus the RI_{Sad} values for the same structures was made, an excellent fit was seen (Fig. 2) but small localised trends were lost from view. Therefore a simple offset plot was used for the sake

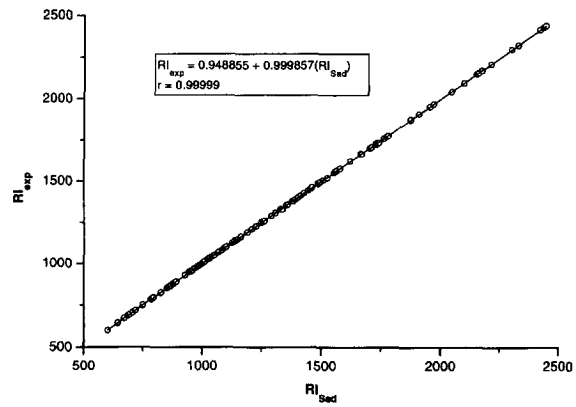

Fig. 2. Linear fit between RI_{exp} and RI_{sad} (134 structures).

of clarity (Fig. 3). Here a small negative dip was apparent and this was fitted unweighted to a second order polynomial, which then gave a predicted offset across the RI_{Sad} range from 600 to 2442 (i.e. to chrysene). The 0.95 prediction limits, being RI dependent, give a search window for each individual structure in the Sadtler dataset incorporated into the 'pseudo-Sadtler' database. The widest (i.e. 'worst case') 0.95 prediction limits for a Sadtler structure were ±3.28 (chrysene; $RI_{Sad}=2442.51$). This tagging with search windows has some similarities to the work of Stowell and Wilson [4].

A similar approach was tried with the Jennings and Shibamoto dataset. Fig. 4 shows an unweighted linear regression fit (93 points rather than 94 due to one outlier) with no obvious localised trends. The RI offset plot showed (Fig. 5) again that there is a slight

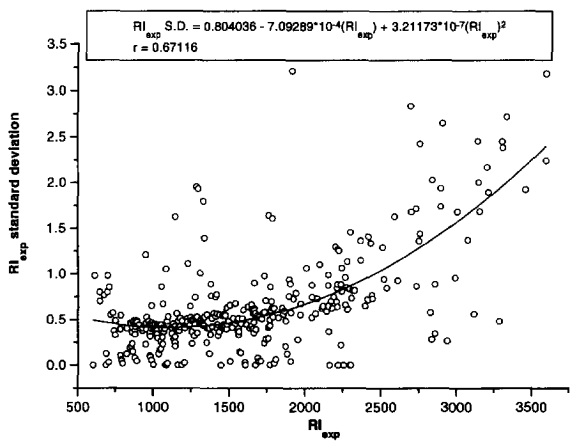

Fig. 1. RI_{exp} standard deviation, vs. RI_{exp} (355 structures).

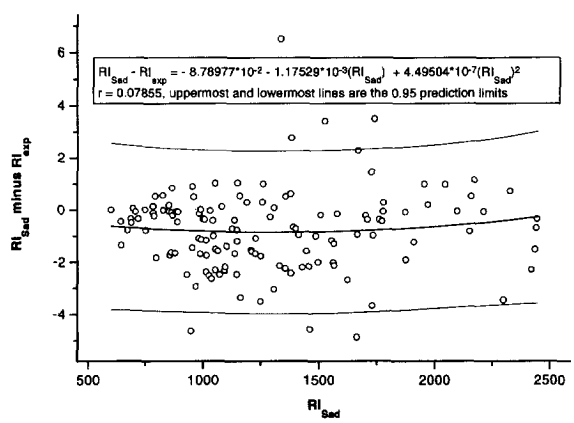

Fig. 3. RI_{sad} minus RI_{exp}, vs. RI_{sad} (134 structures).

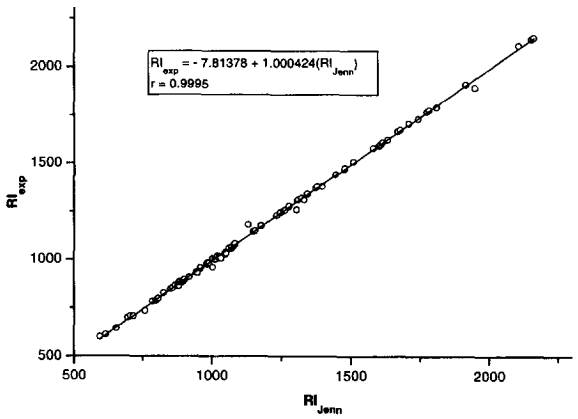

Fig. 4. Linear fit between RI_{exp} and RI_{Jenn} (93 structures).

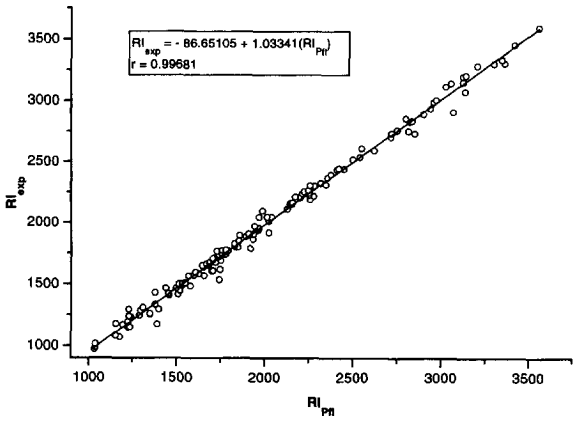

Fig. 6. Linear fit between RI_{exp} and RI_{Pfl} (145 structures).

trend that needs to be compensated for. An unweighted second-order polynomial fit was used to calculate offsets for all of the RI_{Jenn} values (Fig. 4). The widest 0.95 prediction limits for a Jennings and Shibamoto dataset structure were ±25.45 (ethyl oleate; RI_{Jenn}=2155).

The end result of this work was the creation of a 'pseudo-Sadtler' database containing 1872 entries derived from the Sadtler 8 °C/min subset (RI_{Sad}), 357 entries from the presented work (RI_{exp}) and 1186 entries from the Jennings and Shibamoto 'flavours' collection (RI_{Jenn}), which after elimination of duplicates gave a total of 3135 unique entries. The search windows are considerably smaller than those of the TIAFT collection [8]. This was achieved using a faster GC programming rate than the Sadtler Library experimental conditions and with helium rather than hydrogen carrier gas.

However not all data compilations gave smaller search windows than the TIAFT collection e.g. the Pfleger toxicological compilation [9]. A sample set of 149 representative structures within this compilation was run under the 'pseudo-Sadtler' conditions and the RI_{exp} values compared with the corresponding RI_{Pfl} values. Four structures were discarded as obvious outliers giving a total of 145 structures. Although the unweighted linear regression fit appears good (Fig. 6), a simple comparison of Fig. 7 compared with either Fig. 3 or Fig. 5 shows that the scatter of the offsets is much larger. If incorporated into the 'pseudo-Sadtler' dataset, the widest search window would be ±104.27 (tiotixene;

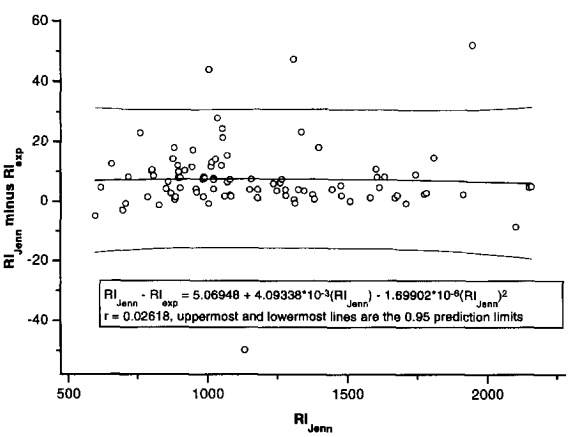

Fig. 5. RI_{Jenn} minus RI_{exp}, vs. RI_{Jenn} (93 structures).

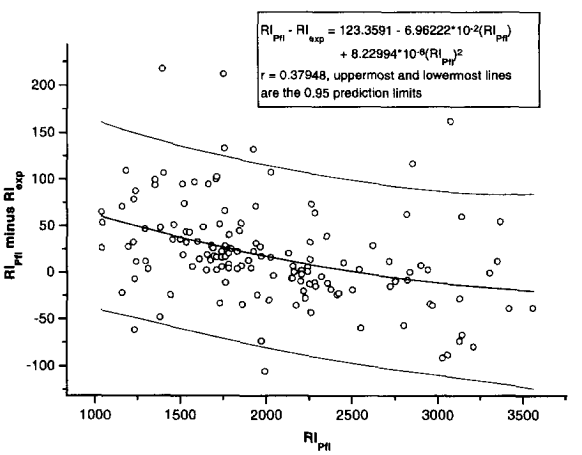

Fig. 7. RI_{Pfl} minus RI_{exp}, vs. RI_{Pfl} (145 structures).

RI_{Pfl}=3555), a clear deterioration compared with the previous two datasets. The reason is probably that the Pfleger compilation [9] is dominated by measurements on a 'Chromosorb G HP 100–120 mesh coated with 5% OV-101' packed column rather than on a capillary column and the retention indices are not comparable (except crudely) due to the different surface chemistries of the two column technologies. Simple linear regression between packed and capillary column RI data can allow the quick conclusion, mostly without statistical qualification, that these two types of RI are compatible [5].

4. Conclusions

This calibration approach is purely empirical and has no thermodynamic [10] or quantitative structure–retention relationship (QSRR) adjustments [11]. However it provides an approach to salvaging some capillary column GC RI compilations by merging them into a 'pseudo-Sadtler' database and has the advantage that each incorporated structure is tagged with a statistically derived search window. When identical structures arise in this 'pseudo-Sadtler' database then the entry with the larger search window can be deleted and so an avenue for continual improvement is provided.

Using this approach a capillary column RI database containing 3135 unique structures was built, which appears to be the largest as yet reported.

References

[1] Y. Sun, A. Huang, R. Zhang and L. He, J. Chromatogr., 648 (1993) 395.
[2] The Sadtler Standard Gas Chromatography Retention Index Library, Sadtler Research Laboratories, Philadelphia, PA, USA, 1984.
[3] W. Jennings and T. Shibamoto, Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Academic Press, New York, 1980.
[4] A. Stowell and L. Wilson, J. Forensic Sci., 32 (1987) 1214.
[5] M. Japp, R. Gill and M.D. Osselton, J. Forensic Sci., 32 (1987) 1574.
[6] H. van den Dool and P.D. Kratz, J. Chromatogr., 11 (1963) 463.
[7] L. Weber, J. High Resolut. Chromatogr. Chromatogr. Commun., 9 (1986), 446.
[8] Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on Packed or Capillary Columns with Dimethyl Stationary Phase, VCH, Weinheim, Germany, 1992.
[9] K. Pfleger, H.H. Maurer and A. Weber, Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, VCH, Weinheim, 2nd ed., 1992.
[10] Z. Hu and H. Zhang, J. Chromatogr. A, 653 (1993) 275.
[11] R.H. Rohrnaugh and P.C. Jurs, Anal. Chem., 60 (1988) 2249.
[12] J.A. Garcia Dominguez and J.M. Santiuste, Chromatographia, 32 (1991) 116.
[13] W.J. Orvis. EXCEL 4 for scientists and engineers, Sybex, Almeda, USA, 1993.