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A Simple Approach to the Interlaboratory Transfer of Drug Retention Indices Determined by Temperature Programmed Capillary Gas Chromatography

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ABSTRACT: Regression analysis of drug retention index (rI) data common to three independent published data bases, and an "in-house" data base, showed excellent interlaboratory correlations for rIs determined by temperature programmed capillary gas chromatography using nonpolar fused silica columns. Satisfactory interlaboratory transfer of rI data was shown to be feasible if appropriate linear regression equations were used to convert published rI data to corresponding in-house data.

KEYWORDS: toxicology, chromatographic analysis, drug retention indices, capillary gas chromatography, interlaboratory data transfer

The use of capillary gas chromatography for both qualitative and quantitative analysis of drugs is rapidly increasing in toxicological and forensic science laboratories worldwide. The resolution, reproducibility, and speed of analysis obtained with this technique are largely responsible for its widespread use, while recent advances in capillary gas chromatographic hardware have accelerated the rapid conversion to capillary methodology in laboratories previously employing packed columns only. Of special importance in this respect is the availability of fused silica (FS) columns, which are not only highly inert, but also robust enough to be easily manipulated by the novice chromatographer [*1*]. The use of such columns for drug analysis has led to several recent reports emphasizing the precision with which Kovats retention indices [*2*] can be determined for drugs chromatographed on nonpolar FS columns, and the use of these indices has been advocated for drug screening methods, using temperature programmed capillary gas chromatography [*3–5*].

Although extensive compilations of Kovats retention indices (rIs) have been made for drugs chromatographed on packed columns [*6, 7*], at the time of writing, we were unaware of any comprehensive drug rI data base compiled using capillary methodology. Furthermore, on inspection of the published data bases, it became clear that packed column rI data for many drugs differed greatly from corresponding data determined on capillary columns. Such differences have been previously recognized and could be due largely to the greater inertness of the fused silica column compared to the packed column support material. There

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is, therefore, a need for more drug rI data relating specifically to FS capillary systems. Some small independent "in-house" rI data bases have been established for drugs run on similar FS capillary columns having the same nonpolar methylsilicone stationary phase [*3–5,8*], but the lack of standardization of other gas chromatographic parameters appeared to preclude the incorporation of these independent data sets into a single more comprehensive rI library.

We have recently compiled our own in-house data base consisting of rI values for 77 drugs chromatographed on bonded phase methylsilicone capillary columns. In this paper we describe the correlation achieved between our own rI data and that found in 3 independent drug rI data bases. This correlation was investigated as a possible means by which our data base could be greatly extended, while avoiding direct determination of rIs for a large number of additional drugs.

Materials and Methods

Instrumentation

A Hewlett-Packard (Avondale, Pennsylvania) Model 5890 gas chromatograph equipped with a flame ionization detector and HP split/splitless injector system was used. The injector was used exclusively in the split mode with a split ratio of approximately 1 : 100. Injector and detector temperatures were 250 and 300°C, respectively. The standard temperature program used for drug screening was 8°C/min from 120 to 270°C, followed by a second ramp at 25°C/min to 300°C, with a 5-min hold at 300°C. A Spectra-Physics SP4270 computing integrator (San Jose, California) was used to record retention times to the nearest second.

Columns and Chromatographic Conditions

Two 12-m by 0.22-mm inside diameter (ID) FS BP1 (methylsilicone-bonded phase) capillary columns were used in this study and were obtained from Scientific Glass Engineering Pty Ltd, Victoria, Australia. Each had a film thickness of 0.25 μm. Helium was used as carrier gas at a linear velocity of 31 cm/s (at 120°C).

Drug and Hydrocarbon Standards

Drug standards used for rI determinations were dissolved in methanol to give solutions of 1.0 mg/mL. Standard mixtures of normal paraffins were prepared in hexane using C10–C20, C22–C26, C28, C30, C34, and C36. Concentrations of the individual hydrocarbons ranged from 1 to 4 mg/mL. Volumes of drug or hydrocarbon solutions or both injected ranged from 0.5 to 2 μL.

Retention Index Calculation

Retention indices were determined by linear interpolation between consecutive hydrocarbon standards as described previously [*3,9*], after coinjection of the drugs with the hydrocarbon standards (internal standardization), or by external standardization using hydrocarbon retention data obtained on the same day as drug rIs were determined.

Results and Discussion

Both the run-to-run and day-to-day standard deviations from the mean drug rIs determined in our laboratory were usually of the order of ±1 rI unit. Greater deviations were observed only when peaks were severely degraded (as a result of column overloading, for example) or when interpolation between widely separated hydrocarbons was performed. For

example, because C31, C32, and C33 were unavailable to us at the time this study was carried out, rIs for strychnine ($\bar{x} \pm SD = 3109 \pm 4$) were determined by interpolation between C30 and C34. No significant differences were seen between rI values determined on the two different BP1 columns or by internal and external standardization.

Retention indices determined in our laboratory for 77 drugs of forensic and toxicological interest are listed in Table 1 (Data Base 1) together with corresponding data from published rI data bases established in 3 independent laboratories (Data Bases 2, 3, and 4) using FS capillary columns similar to those used in our laboratory. Data Bases 2, 3, and 4 have a total of 166, 173, and 33 drug rI values, respectively, but only those with corresponding values

TABLE 1—*Interlaboratory comparison of retention indices.*[a]

Drug	Retention Indices			
	Data Base 1	Data Base 2	Data Base 3	Data Base 4
Dexamphetamine	1116	1118 (+4)	1111 (+5)	
Phentermine	1152		1138 (−3)	
Methamphetamine	1175	1173 (0)	1161 (−3)	
Mephentermine	1244	1243 (+1)	1236 (—4)	
D-Norpseudoephedrine	1303			
Phenylpropanolamine	1304	1308 (+5)	1287 (−4)	
Nicotine	1331	1326 (−4)	1315 (−3)	1311 (+2)
Ephedrine	1350	1350 (+1)		
Pseudoephedrine	1350	1360 (+11)		
Diyclohexylamine	1408			
Diethylpropion	1483		1470 (−1)	
Etenzamide	1544			
Paracetamol	1636	1631 (−4)		1626 (−6)
Phenacetin	1651			
Mescaline	1664	1663 (0)	1657 (+10)	
Amobarbital	1697	1697 (+1)		1690 (−5)
Methylphenidate	1705		1695 (+7)	1704 (+3)
Pethidine	1731	1730 (0)		
Quinalbarbitone	1763	1769 (+6)		1763 (−3)
Caffeine	1775	1768 (−6)	1749 (−8)	1764 (−1)
4-bromo-2,5-dimethoxyamphetamine	1782			
Diphenhydramine	1852	1849 (−3)		
Lignocaine	1857	1854 (−3)	1842 (+3)	1852 (−5)
Phencyclidine	1879		1860 (+1)	
Theophylline	1917	1917 (0)		
Phenobarbital	1924	1928 (+4)		1922 (+1)
Orphenadrine	1924	1924 (0)	1915 (+11)	
Flufenamic acid	1932			
Procaine	1991		1978 (+8)	1990 (−3)
Clonidine	2023			
Dicyclomine	2095	2091 (−4)	2080 (+7)	
Methaqualone	2119	2115 (−4)	2096 (−1)	2117 (−10)
Dextromethorphan	2121	2116 (+5)	2097 (−2)	
Methadone	2137	2131 (−6)	2121 (+7)	2135 (+3)
Hyoscyamine	2168	2174 (+6)	2146 (+1)	
Atropine	2169		2147 (+1)	2169 (0)
Cocaine	2179	2175 (−4)	2161 (+5)	2176 (+6)
Propoxyphene	2181	2178 (−3)	2165 (+7)	2181 (+6)
Amitryptiline	2181	2179 (−2)	2162 (+4)	
Pindolol	2199			
Trimipramine	2203			
Benzhexol	2236	2226 (−10)		

TABLE 1—*Continued*

Drug	Retention Indices			
	Data Base 1	Data Base 2	Data Base 3	Data Base 4
Triprolidine	2242		2224 (+6)	
Pentazocine	2262		2246 (+8)	
Timolol	2262			
Phenytoin	2289	2289 (−1)		2282 (−5)
Oxazepam	2300	2293 (−8)	2271 (−4)	2294 (−1)
Lynoestriol	2319			
Phenylbutazone	2345	2344 (−2)		
Codeine	2353	2348 (−5)	2323 (−5)	2355 (+3)
Dothiepin	2364			
Azatadine	2368			
Morphine	2399	2406 (+6)	2367 (−6)	2396 (+3)
Diazepam	2409	2404 (−6)	2383 (0)	2407 (−2)
Mebhydrolin	2449			
Nordiazepam	2462	2459 (−4)		2457 (+2)
Δ^9-Tetrahydrocannabinol	2466			
Chlorpromazine	2480	2474 (−7)	2452 (−1)	2481 (+3)
Acetylcodeine	2484	2480 (−5)		
Disopyramide	2489			
Thebaine	2492			
O^6-Monoacetylmorphine	2495			
Metoclopramide	2602			
Heroin	2607	2605 (−3)	2581 (+2)	2605 (−1)
Ethinyloestradiol	2635			
Prednisone	2651			
Trifluoperazine	2666	2662 (−5)	2641 (+4)	
Nitrazepam	2720	2714 (−7)		
Flurazepam	2769	2763 (−7)	2741 (+3)	
Chlordiazepoxide	2774	2778 (+2)	2742 (−2)	
Papaverine	2814			
Dextromoramide	2932			
Triazolam	3017	3008 (−11)		
Fluphenazine	3032			
Thioridizine	3105	3117 (+10)	3080 (+10)	
Strychnine	3109	3109 (−2)	3058 (−16)	
Noscapine	3135	3168 (+31)		

[a]See Table 2 for references to the four data bases and for details of the conditions used for their compilation. The numbers in parentheses are the absolute deviations of the tabulated rI values from the appropriate regression lines given in Table 3.

determined in our own laboratory have been included in Table 1. Table 2 lists the columns and analytical conditions used for the establishment of each data base.

The excellent correlation between the 4 independent data bases is illustrated by the linear regression data in Table 3. Interlaboratory correlation of this degree was unexpected, since some important chromatographic conditions were not standardized (for example, programming rate, carrier flow rate, and injection mode) and some wide interlaboratory rI differences were seen for many drugs. However, in view of the correlations observed, we were led to examine the usefulness of the combined data sets (including a total of 265 drugs) as reference data for drug screening in our laboratory. To this end, we calculated the degree to which the individual published rI values deviated from the appropriate regression lines (described by the equations in Table 3). Such deviations are presented in parentheses in Table 1 and were calculated in the following manner. For each data base comparison, the difference between published y values and those derived by solving the appropriate regression equation were

TABLE 2—*Analytical conditions used in the determination of each of the four data bases listed in Table 1.*

Data Base	Reference	Column Type	Column ID, mm	Phase Thickness, μm	Injection Mode	Temperature Program	Carrier Gas and $\bar{\mu}$
1	This study	FS SGE BP1 12m	0.22	0.25	split	120 $\xrightarrow{8°/min}$ 270°C 270 $\xrightarrow{25°/min}$ 300°C	He 31 cm/s at 120°C
2	*5*	FS J&W DB1 15m	0.22	0.25	split	120 $\xrightarrow{8°/min}$ 280°C	He $\bar{\mu}$ not given
3	*4*	FS J&W SE-30 15m	0.25	0.25	split	100 $\xrightarrow{5°/min}$ 295°C	He 45 cm/s at 100°C
4	*3*	FS HP Ultra 12m	0.2	0.33	splitless	50 $\xrightarrow{20°/min}$ 300°C	He 80 cm/s No temp. given

TABLE 3—*Regression analysis of retention index data from the four independent data bases listed in Table 1.*

Data Bases Compared (x vs y)	n	Regression Line	r
1 vs 2	44	$y = 1.002 - 4$	0.9999
1 vs 3	37	$y = 0.988x + 3$	0.9999
1 vs 4	22	$y = 1.009x + 22$	0.9999

calculated, using known x values from Data Base 1. These differences were then used to determine the range of values that should be searched to identify an unknown peak when using our standard chromatographic conditions and using the published data bases as reference material. The differences between the published and derived rI values determined in the above way were such that for Data Base 2, 93% of the derived rI values fell within a ±10-unit range of the published values and 98% within a ±15-unit range. For Data Base 3, the corresponding percentages were 94 and 97%, and for Data Base 4, all derived rI values fell within the ±10-unit range. The mean deviations of derived rI values from the published values were 5, 5, and 3 for Data Bases 2, 3, and 4, respectively. These deviations were considered acceptable for a screening method. If, for example, Data Base 2 (containing a total of 166 drugs) was being searched, allowing for an error factor of ±10 units, the average number of drugs bracketed by this 20-unit range would be 2, with the maximum number being 7. For the ±15-unit error factor, the corresponding numbers of drugs would be 3 and 10. Similar results were found for Data Base 3, containing 173 drugs.

An attempt was made to improve the correlation between Anderson and Stafford's data (Data Base 3) [*4*] and the data generated in our laboratory, by employing the same temperature program and carrier flow used by these workers. Although this resulted in rI values considerably closer to those published by Anderson and Stafford, the correlation between the in-house and published data was not improved sufficiently to allow use of a reduced error factor when searching Data Base 3. It appears, therefore, that strict interlaboratory standardization of gas chromatographic conditions may not be necessary, if the above approach to the interlaboratory transfer of rI data is followed.

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