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# Interlaboratory Reproducibility of Retention Indices in Capillary Gas Chromatography

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**ABSTRACT:** Because of the temperature-dependent behavior of Kovat's retention indices (RI), based on straight-chain alkane homologues and a liquid phase such as SE-30, OV-1, or methyl silicone, the interlaboratory reproducibility of these RIs determined on capillary columns is comparable to those determined on packed columns. Other homologue series investigated—including diisopropyl-*n*-alkylamines (DIPA), tri-*n*-alkylamines (TAA), and 1-nitro-*n*-alkanes (NIA)—showed the same phenomenon. However, by using a carefully selected reference drug mixture, a dramatic gain in the interlaboratory reproducibility of RI values can be obtained, even under vastly different operational conditions. This allows a search window of ±25 RI units for capillary methyl silicone columns, which is much better than the ±60 RI units that must be applied when using alkane or substituted alkane homologues.

**KEYWORDS:** toxicology, chromatographic analysis, retention indices, capillary gas chromatography, substance identification, drugs, systematic toxicological analysis, interlaboratory survey

Gas chromatography (GC) on packed SE-30 or OV-1 columns is a recommended method for drug screening in systematic toxicological analysis (STA) [*1*]. Data are available for over 1700 relevant substances in the form of retention indices (RI) based on *n*-alkanes [*2*]. However, the interlaboratory reproducibility of RIs is relatively low, with a standard deviation of 20 RI units, which results in a search window of ±60 RI units when identifying unknown substances.

Several investigators found that RIs determined on capillary columns with a methyl silicone phase are comparable to those obtained on packed columns [*3–5*]. Yet, despite the higher separation power of capillary columns, the same search window has to be considered. This is primarily because capillary GC for screening purposes is almost always done in a temperature-programed mode, and since RIs are temperature dependent, they will be determined by the elution temperature, which is in turn a function of the temperature program selected, the column characteristics, and the flow rate of the carrier gas. Theoretically, these problems may be circumvented by using a fixed-temperature program on a given column in a given instrument, but this is, of course, totally unrealistic for interlaboratory studies.

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For a given stationary phase such as methyl silicone, the interactions between the stationary phase and the nonpolar *n*-alkanes may be different from interactions between the stationary phase and substituted alkanes or drugs generally encountered in toxicology. Therefore, we investigated whether RIs determined with reference mixtures of substituted alkanes or drugs would be less susceptible to differences in elution temperature. If so, these mixtures could then be substituted for the *n*-alkanes as the basis for calculating RIs and thus could provide better interlaboratory reproducibility.

## Materials and Methods

### *Instrumentation*

A Hewlett-Packard (Avondale, PA) Model 5880 gas chromatograph, equipped with a flame ionization detector, split/splitless injector system, and an automatic injector was used. The injector and detector temperature were 275 and 310°C, respectively. A Scientific Glass Engineering (SGE) (Victoria, Australia) DB-1 column with a length of 25 m and a film thickness of 3.0 μm was used. The flow rate of the helium carrier gas was 9 mL/min and the temperature program was as follows: 4 min at 135°C; increased 13°/min to 200°C, 8°/min to 240°C, and 6°/min to 312°C; 6 min at the final temperature. An aliquot of 3 μL of the individual substance, a concentration of approximately 0.1 mg/mL in ethyl acetate, or a reference mixture was injected.

### *Reference Mixtures*

The following mixtures were used:

1. n-Alkanes: $C_{10}$ through $C_{26}$, $C_{28}$, $C_{30}$, and $C_{32}$, each 0.2 mg/mL in hexane [*2*].
2. Diisopropyl-*n*-alkylamines (DIPA): $C_{17}$, $C_{18}$, and $C_{20}$, each 0.1 mg/mL in ethyl acetate.[2]
3. Tri-*n*-alkylamines (TAA): $C_6$ through $C_5$, each 0.2 mg/mL in ethyl acetate [*6*].
4. 1-Nitro-*n*-alkanes (NIA): $C_{16}$ through $C_{18}$, each 0.2 mg/mL in ethyl acetate [*7*].
5. Drug mixture for acidic and neutral drugs according to Table 1, each 0.1 mg/mL in ethyl acetate.
6. Drug mixture for basic drugs according to Table 1, each 0.1 mg/mL in ethyl acetate.

The selection of substances for the drug mixtures was done on the basis of their general availability and stability. Furthermore, the substances had to cover a wide and toxicologically relevant RI range. Also, as it is common practice in toxicology to carry out pH-dependent extractions prior to chromatography, two mixtures were composed, one for acidic and neutral drugs (Mixture A) and one for basic and neutral drugs (Mixture B). Table 1 also provides the RI reference values [RI(K)] of the various drugs, derived from those given in Ref *1*. These values were then used to calculate the retention indices [RI(DM)] of other substances, as outlined below.

The hexane and ethyl acetate, both of analytical grade, and the straight-chain alkanes were obtained from Merck (Darmstad, FRG). The tri-*n*-alkylamines were obtained from Merck (Darmstad, FRG) and from Eastman Kodak (Rochester, NY). The 1-nitro-*n*-alkane homologues were a gift from Dr. M. Bogusz (Institut für Rechtsmedizin, Heidelberg, FRG) and the diisopropyl-*n*-alkylamine homologues were a gift from Prof. M. Donike (Institut für Biochemie, Cologne, FRG). All the drug samples were of pharmaceutical quality and were used as obtained.

[2]Donike, M., Institut für Biochemie, Cologne, West Germany, personal communication, 1981.

TABLE 1—*Drug mixtures for calculating retention indices [RI(DM)], with their reference RI values.*[a]

| For Acidic and Neutral Drugs | | For Basic and Neutral Drugs | |
|---|---|---|---|
| Substance | Reference Value, RI(K) | Substance | Reference Value, RI(K) |
| Ethosuximide | 1200 | Amphetamine | 1110 |
| Ethinamate | 1360 | Ephedrine | 1365 |
| Barbital | 1490 | Benzocaine | 1545 |
| Aprobarbital | 1610 | Methylphenidate | 1725 |
| Secobarbital | 1780 | Diphenhydramine | 1860 |
| Phenobarbital | 1940 | Tripelennamine | 1980 |
| Heptabarbital | 2050 | Methaqualone | 2140 |
| Primidone | 2180 | Trimipramine | 2215 |
| Phenylbutazone | 2365 | Codeine | 2375 |
| Di(2-ethylhexyl) phthalate | 2500 | Desmethyldiazepam | 2485 |
| Prazepam | 2645 | Prazepam | 2645 |
| Clonazepam | 2810 | Papaverine | 2820 |
| | | Haloperidol | 2945 |
| | | Strychnine | 3120 |

[a]The solutions are in ethyl acetate; the RI reference values [RI(K)] are based on Ref *1*.

*Interlaboratory Survey*

The participants of the interlaboratory survey were all using fused silica capillary columns of their own choice, with an inner diameter of 0.53 mm and a chemically bonded methyl silicon phase. The participants in the study, with the sets of conditions used, are listed here:

1. H. J. Battista, Institut für Gerichtliche Medizin, Innsbruck, Austria, used a J&W DB-1 column with a length of 15 m and a film thickness of 1.5 μm. The flow rate of the nitrogen gas was 10 mL/min and the temperature program was as follows: 2 min at 130°C; increased 10°/min to 280°C for acidic and neutral drugs and to 290°C for basic drugs; 12 min at the final temperature.

2. J. P. Franke, University Center for Pharmacy, Groningen, The Netherlands, used a SGE DB-1 column with a length of 25 m and a film thickness of 3.0 μm. The flow rate of the helium carrier gas was 9 mL/min and the temperature program was as follows: 4 min at 135°C; increased 13°/min to 200°C, 8°/min to 240°C, and 6°/min to 312°C; 6 min at the final temperature.

3. G. Machata, Institut für Gerichtliche Medizin, Vienna, Austria, used a HP OV-1 column with a length of 10 m and a film thickness of 1.0 μm. The flow rate of the helium carrier gas was 5 mL/min and the temperature program was as follows: increased 8°/min from 100 to 260°C; 15 min at the final temperature.

4. V. J. McLinden, Government Chemical Laboratories, Perth, Australia, used a HP OV-1 column with a length of 10 m and a film thickness of 2.6 μm. The flow rate of the nitrogen gas was 10 mL/min and the temperature program was as follows: increased 10°/min from 100 to 280°C; 10 min at the final temperature.

5. M. D. Osselton, Home Office Central Research Establishment, Aldermaston, England, used a J&W DB-1 column with a length of 28 m and a film thickness of 1.5 μm. The flow rate of the nitrogen gas was 10 mL/min and the temperature program was as follows: (*a*) for acidic and neutral drugs: 3 min at 120°C; increased 10°/min to 200°C, 7°/min to 250°C, and 3°/min to 300°C; 10 min at the final temperature; and (*b*) for basic drugs: 3 min at 120°C; increased 12°/min to 200°C, 9°/min to 250°C, and 3°/min to 300°C; 10 min at the final temperature.

*Retention Index Calculation*

The RIs were calculated for temperature-programed conditions by linear interpolation, either between consecutive homologues (*n*-alkanes, diisopropylalkylamines, nitroalkanes, or trialkylamines) or between bracketing drugs in one of the drug reference mixtures. In the case of diisopropyl-*n*-alkylamines (DIPA), tri-*n*-alkylamines (TAA), and 1-nitro-*n*-alkanes (NIA), the obtained RI was converted to an *n*-alkane-based RI by the equations given in Table 2. In the case of drug mixtures, the RI reference values (Table 1) were plotted versus the observed retention times. For basic and neutral drugs this resulted in the graph shown in Fig. 1. RI(DM)s for other drugs were then determined from this graph by linear interpolation. For Drug Mixture A, a similar, almost linear plot was obtained.

For determination of the temperature-dependent effects under isothermal conditions, the RIs were determined by logarithmic interpolation between the bracketing homologues coinjected with the drug under study.

## Results and Discussion

*Kovat's Retention Indices*

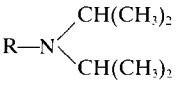
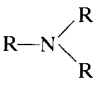
In isothermal runs, but at different oven temperatures, the RI(K)s were measured for three drugs: codeine, methaqualone, and morphine. The values obtained at the capillary column ($RI_{cap}$) were then compared with the literature value in Ref *1* ($RI_{lit}$), and the differences, $RI_{cap} - RI_{lit}$, were plotted. Figure 2 shows that there is an almost linear relationship between RI and the oven temperature over a wide temperature range. With methaqualone and codeine, some small deviations from the straight line were observed because of overlapping with one of the coinjected straight-chain alkanes.

It can also be seen that the slopes of the lines are substance dependent and that they are relatively steep. For codeine, for example, an increase in RI of about 100 units was observed between 190 and 270°C. Apparently, the temperature is the main cause of variability.

*Retention Indices Based on Other Reference Series*

In recent years various substituted alkane series have been suggested for RI calculations, mainly to achieve better detectability by such selective detectors as nitrogen phos-

TABLE 2—*Alkane homologues for RI calculations.*

| Homologues/Interrelationship Reference | Abbreviation | RI designation | Structure[a] |
|---|---|---|---|
| *N*-Alkanes<br>Kovats, 1958 [*2*] | | RI(K) | R—H |
| Diisopropylalkylamines<br>RI(K) = RI(D) + 584<br>Donike, 1981[b] | DIPA | RI(D) |  |
| Tri-*n*-alkylamines<br>RI(K) = RI (T) × 2.79 + 69<br>Watts and Simonick, 1987 [*6*] | TAA | RI(T) |  |
| Nitroalkanes<br>RI(K) = RI(N) × 1.02 + 431<br>Aderjan and Bogusz, 1988 [*7*] | NIA | RI(N) | $R—NO_2$ |

[a] $R = C_nH_{2n+1}$

[b] Donike, M., Institut für Biochemie, Cologne, West Germany, personal communication, 1981.

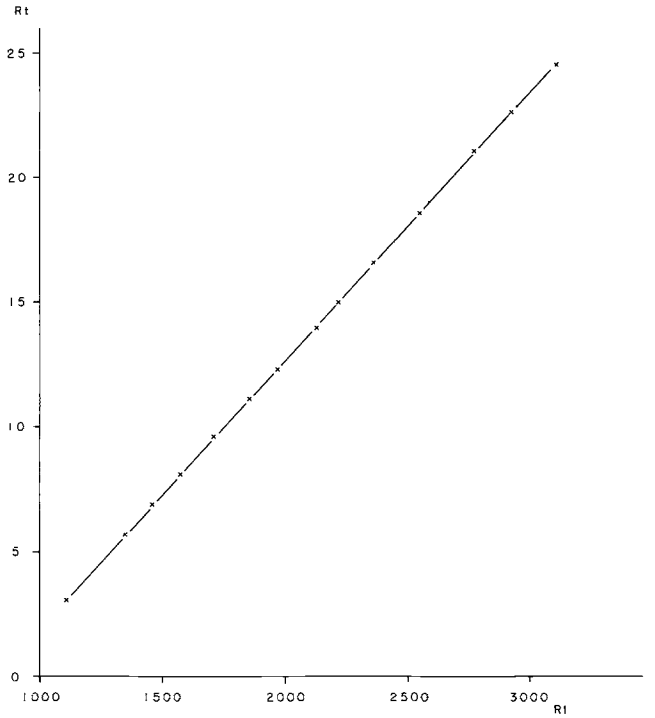



FIG. 1—*Retention times versus Kovat's retention indices for reference Drug Mixture B in a temperature-programed run on a capillary DB-1 column.*

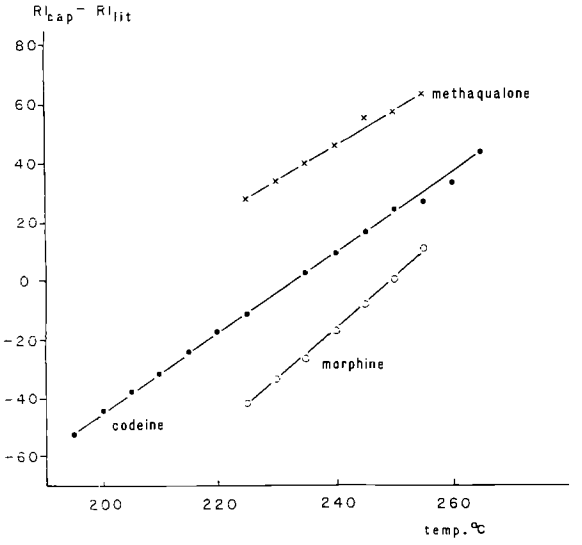



FIG. 2—*Temperature dependence of Kovat's retention indices, RI(K). The determinations were made in isothermal runs on a capillary DB-1 column.*

phorus detectors (NPDs) and electron capture detectors (ECDs). A selection is listed in Table 2, together with the *n*-alkanes. We have assessed their usefulness for improving the reproducibility of RIs in GC. Analyses were done in isothermal runs and with codeine as the test compound. The results are depicted in Fig. 3 and tabulated in Table 3.

It is clear that RI values determined with all four alkane series are highly temperature dependent and that the increase in RIs with temperature is virtually the same for all alkane series. However, when the drug mixture is used as a reference series, the RI(DM)s thus obtained are much less temperature dependent. Apparently the changes in the retention processes due to differences in temperature and flow are much better compensated for with the drug mixture than with the alkane series.

*Interlaboratory Variability of Retention Indices Determined Using Drug Mixtures*

As the drug mixtures seemed to be much better for determining temperature differences in temperature-programed runs, an interlaboratory survey was carried out between 5

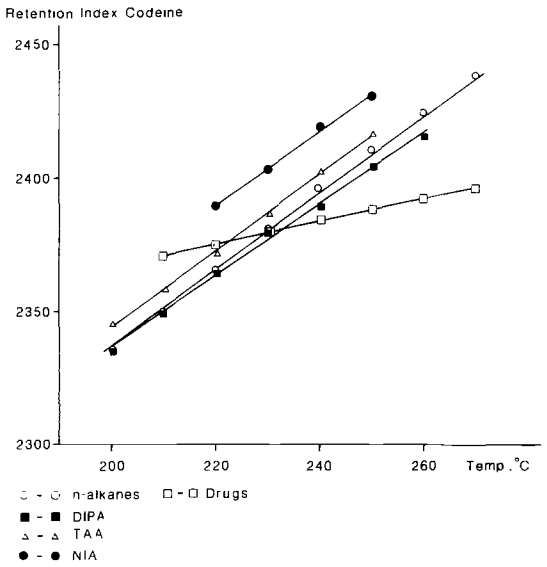



FIG. 3—*Temperature dependence of retention indices of codeine, using different reference standards. The determinations were made in isothermal runs on methyl silicone capillary columns.*

TABLE 3—*Impact of temperature on the retention index of codeine, determined by different reference substances.*

| Reference Substances | RI(K) for Codeine at 230°C | RI Increase, units/°C |
|---|---|---|
| *n*-Alkanes | 2382 | 1.42 |
| Diisopropyl-*n*-alkylamines (DIPA) | 2377 | 1.32 |
| Tri-*n*-alkylamines (TAA) | 2387 | 1.40 |
| Nitroalkanes (NIA) | 2403 | 1.40 |
| Drug Mixture B | 2380 | 0.43 |

laboratories, in which each participant determined RI(DM) values for 30 acidic and neutral substances and 30 basic substances, using Drug Mixtures A and B, respectively, as references. Participants were asked to use their choice of a wide-bore capillary methyl silicone column in a temperature-programed run of their liking. No further conditions were prescribed. The results indicated that there was no systematic difference in the behavior of acidic and neutral drugs on the one hand and basic drugs on the other. Therefore, data from both categories were combined and the distribution of the standard deviations observed for the individual drugs was plotted. These data are depicted in Fig. 4, which shows that a mean standard deviation of 8 RI(DM) units was found. This compares very favorably with the interlaboratory standard deviation of 20 found with alkane homologues [*3*,*5*].

A more detailed study of the data set revealed that the capillary columns with a thin film gave somewhat lower values relative to the mean, whereas the long column with the thick film gave somewhat higher values.

Additional studies, including other selections of drugs as well as other institutes, will be needed to determine whether the drug mixtures are indeed a better alternative for obtaining better interlaboratory reproducibility of GC RIs of toxicologically relevant drugs. If so, it would be advantageous to start building up a large database of RI(DM) values on capillary columns.

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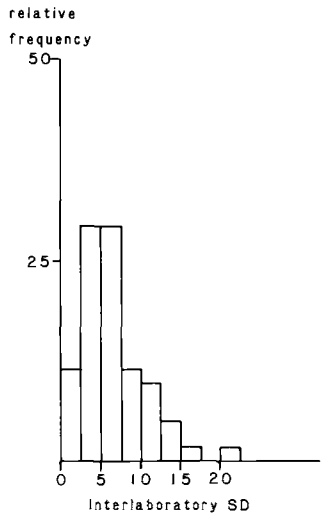


FIG. 4—*The distribution of standard deviations of RI(DM)s for the individual drugs in the interlaboratory investigation.*

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