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Note

Nitroalkanes as a multidetector retention index scale for drug identification in gas chromatography

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The need for the standardization of retention data has been obvious from the first application of gas chromatograpy (GC). The retention index (RI) system based on homologues of *n*-alkanes proposed by Kováts[1] has been widely accepted in various fields of chromatographic analysis[2–4] and also in analytical toxicology, where the identification of unknown substances is particularly important. Compilations and libraries of RI values of toxicologically relevant compounds examined on packed columns have enabled the interlaboratory exchange and use of data[5–8]. More recently, RI values of various drugs determined on capillary columns have been shown to be comparable with those on packed columns[9–15] with only slight deviations.

Under normal working conditions, selective GC detectors do not respond to *n*-alkanes. Alternative standardization systems for GC data have therefore been developed, with the use of alkanoic acid methyl esters[16,17], steranes[18], 2-alkanones[19], straight chain alcohols[20], *n*-alkyltrichloroacetates[21,22], polycyclic aromatic hydrocarbons[23], dialkylsulphides[24], diisopropylaminoalkanes[8], *n*-alkylbis(trifluoromethyl)-thiophosphinates[25], *n*-bromoalkanes[26,27], *n*-trialkylamines[28,29], fused quinones[30] and *n*-alkylbis(trifluoromethyl)phosphine sulphides[31]. All these systems are not regarded as satisfactory by us, as they were usually designated for a particular detection. Only two series of closely related compounds proposed as an alternative RI scale meet the demands of multiple detectability[25,31], *i.e.* by electron-capture or thermionic detection (ECD or TID). However, the synthesis of these probably very toxic materials does not seem to be simple and is not described in the literature.

In a previous study the applicability and advantages of the lower 1-nitroalkanes (C_1–C_6) as a retention index system for high-performance liquid chromatography (HPLC) was shown in comparison with another scale[32,33]. In the present paper the use and preparation of higher 1-nitroalkanes as an alternative retention index scale in GC, applicable to all common detector systems, is described. To our knowledge, 1-nitroalkanes are the first series of homologues applicable as a retention index scale both for liquid chromatography (reversed-phase HPLC) and for GC with ECD, TID and flame ionization detection.

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MATERIALS AND METHODS

Nitroalkanes

Nitromethane, nitroethane, 1-nitropropane, 1-nitrobutane, 1-nitropentane and 1-nitrohexane were obtained from Fluka (Buchs, Switzerland). Higher homologues were synthesized according to Kornblum *et al.*[34,35] using the following procedure: 7.5 mmol (1.16 g) of silver nitrite were suspended in 3 ml anhydrous diethyl ether in a head-space vial 25 ml in volume covered with aluminium foil and equipped with a small size magnetic stirrer. The mixture was cooled to 4°C. In a second head-space vial a cold solution of 5 mmol of the corresponding *n*-bromo- (or iodo-) alkane in 3 ml of anhydrous diethyl ether was prepared. From this, every 30 min a portion of 0.5 ml was drawn up into a polypropylene syringe and injected into the stirred silver nitrite suspension. Contact with moisture was avoided as far as possible, and during the reaction the products were protected from light. The reaction mixture was kept at 4°C for 36–48 h and then at room temperature for 24–36 h until the reaction was complete. After filtration from the resulting silver halide, the solvent was evaporated with a stream of nitrogen. The 1-nitroalkanes were obtained in a nearly quantitative yield and with greater than 95% purity. No further purification was needed when the starting materials were of analytical grade. However, solid 1-nitroalkanes may be recrystallized from methanol, 4% water in methanol, or from acetone (homologues above C_{20}).

Other reagents and standard substances

n-Alkanes were supplied by Merck (Darmstadt, F.R.G.). Drugs were of analytical grade from various manufacturers. 1-Bromoalkanes (C_7–C_{18}), as well as *n*-alkanols with even carbon numbers (C_{20} C_{30}), were from Fluka. 1-Iodoalkanes, used for the synthesis of 1-nitroalkanes with more than 18 carbon atoms, were prepared from the commercially available *n*-alcohols, red phosporus and iodine[36,37], according to the following procedure: 1 mmol *n*-alkanol, 1 mmol iodine and 0.33 mmol red phosphorus were kept at 160°C for 3 h. The individual iodides were extracted with toluene and the toluene extract was washed with water followed by 0.2 *M* sodium hydroxide solution. After washing with water again, the toluene was partially evaporated. On cooling the remaining toluene extract, the iodoalkanes crystallized in *ca.* 90% of the theoretical yield. After recrystallization from methanol or acetone no further purification was needed for the synthesis of 1-nitroalkanes.

Gas chromatography

Two Shimadzu (Model 9 A and Model 9 AM) gas chromatographs, equipped with ECD, TID (rubidium chloride bead, electrically heated) and FID were used. Fused-silica capillary columns (10 m × 0.53 mm I.D.), coated with CP-Sil 5 CB (methyl polysiloxane, film thickness 5 μm) and with CP-Sil 19 CB (50% phenyl methyl polysiloxane, film 2 μm) were supplied by Chrompack (Middelburg, The Netherlands). The columns were installed directly in the injector using a deactivated glass liner. Helium was used as the carrier gas at flow-rate of 7 ml/min, as in the previous screening toxicological procedures[38]. The gas flows to the detectors were: for FID, air 500 ml/min, hydrogen 50 ml/min; for TID, air 150 ml/min, hydrogen 3.5 ml/min; for ECD, argon–methane (95:5) 330 ml/min. No make-up gas was used.

The samples were analyzed isothermally at 160 and 220°C or using a temperature programme: 2 min at 100°C, then raised at 15°C/min to 200°C, at 10°C/min to 300°C, finally maintained for 8 min at 300°C. The two-step program gives a better approximation to a linear relationship between the k' values of n-alkanes and their carbon numbers than the simple one, and is commonly used in toxicological screening[38].

Calculations of retention indices

The retention indices of the drugs examined were calculated either from an isothermal experiment using

$$\mathrm{RI} = 100n + 100(N - n) \cdot \frac{\log t_R(\mathrm{x}) - \log t_R(n)}{\log t_R(N) - \log t_R(n)} \quad (1)$$

or from a temperature-programmed experiment according to

$$\mathrm{RI} = 100n + 100(N - n) \cdot \frac{t_R(\mathrm{x}) - t_R(n)}{t_R(N) - t_R(n)} \quad (2)$$

where n and N are the carbon numbers of consequentively eluted reference compounds (n-alkanes or 1-nitroalkanes), x is the substance examined and t_R is the net retention time. A linear elution of the reference substances during the temperature-programmed experiment was assumed[4,39]. The column dead time was determined with methane and FID.

RESULTS AND DISCUSSION

The stable, non-explosive 1-nitroalkanes having seven or more carbon atoms were easily prepared from the corresponding 1-bromoalkanes or 1-iodoalkanes by a heterogeneous SN1 reaction. All nitroalkanes gave symmetrical GC peaks. The relationship between the logarithm of k' and the carbon number was linear for 1-nitroalkanes analyzed isothermally on both columns (Fig. 1).

The 1-nitroalkanes are easily detectable with FID, ECD or TID. In the chromatograms of the unpurified 1-nitroalkanes, negligible impurities were found by FID or TID. With ECD, however, a few additional peaks were observed, but these can be distinguished from 1-nitroalkanes. Comparison of the magnitudes of the best detector signal for individual 1-nitroalkanes revealed a ratio of approximately 1:3:6 for FID, TID and ECD, respectively.

The RI values of thirteen selected drugs were determined using the n-alkane and the 1-nitroalkane scale on CP-Sil 5 and CP-Sil 19 columns. The relationship between the adjusted retention times and the carbon numbers of n-alkanes or 1-nitroalkanes under these conditions was approximately linear (Fig. 2). The RI values of the substances examined calculated with both reference systems are listed in Table I. The relationship between the RI values calculated on the two scales was linear for both polysiloxane columns. The data were fitted by regression lines expressed by

$$y = 1.02x + 430 \qquad r = 0.9999 \text{ (CP-Sil 5)} \quad (3)$$

$$y = 1.07x + 530 \qquad r = 0.9997 \text{ (CP-Sil 19)} \quad (4)$$

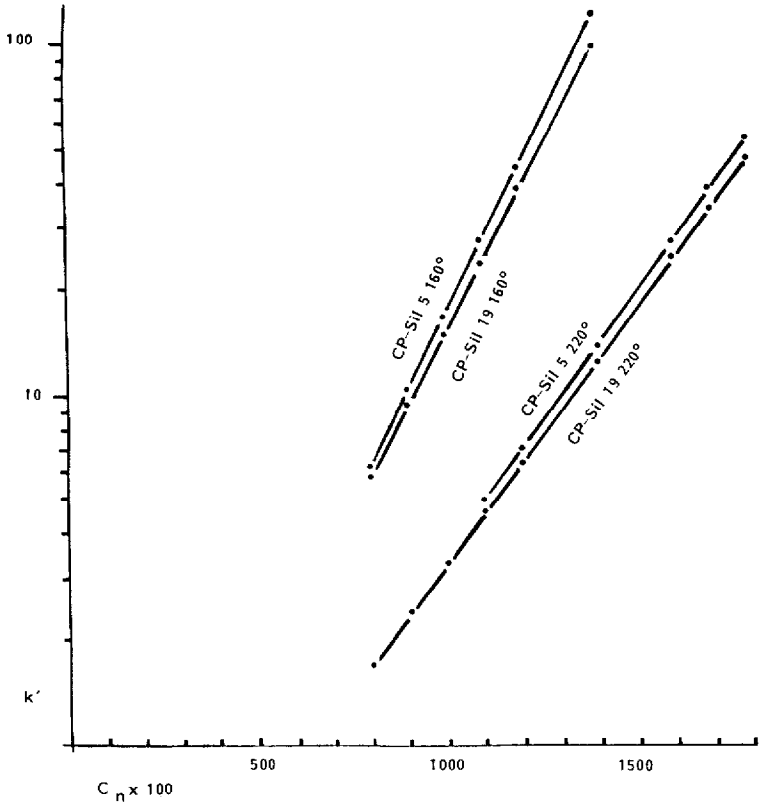

Fig. 1. Relationships between the k' values and the carbon numbers of 1-nitroalkanes at various temperatures on CP-Sil 5 and CP-Sil 19 columns. Semilogarithmic scale.

TABLE I

RETENTION INDEX VALUES OF SUBSTANCES EXAMINED ON CP-SIL 5 AND CP-SIL 19 COLUMNS, RELATIVE TO THE *n*-ALKANES (RI_{Kovats}) AND THE 1-NITROALKANES (RI_{NO_2})

Drug	*CP-SIL 5*		*CP-SIL 19*	
	RI_{Kovats}	RI_{NO_2}	RI_{Kovats}	RI_{NO_2}
Clomethiazole	1245	800	1398	810
Bromdecane	1370	919	1644	1052
Barbital	1495	1047	1828	1208
Aprobarbital	1619	1170	1875	1246
Amobarbital	1711	1253	1943	1313
Caffeine	1809	1341	2143	1527
Diphenhydramine	1869	1420	2025	1405
Parathion	1962	1516	2269	1634
Methaqualone	2152	1685	2466	1815
Amitriptyline	2208	1742	2440	1790
Nordiazepam	2494	2035	3054	2348
Nitrazepam	2744	2271	—	—
Strychnine	3146	2660	—	—

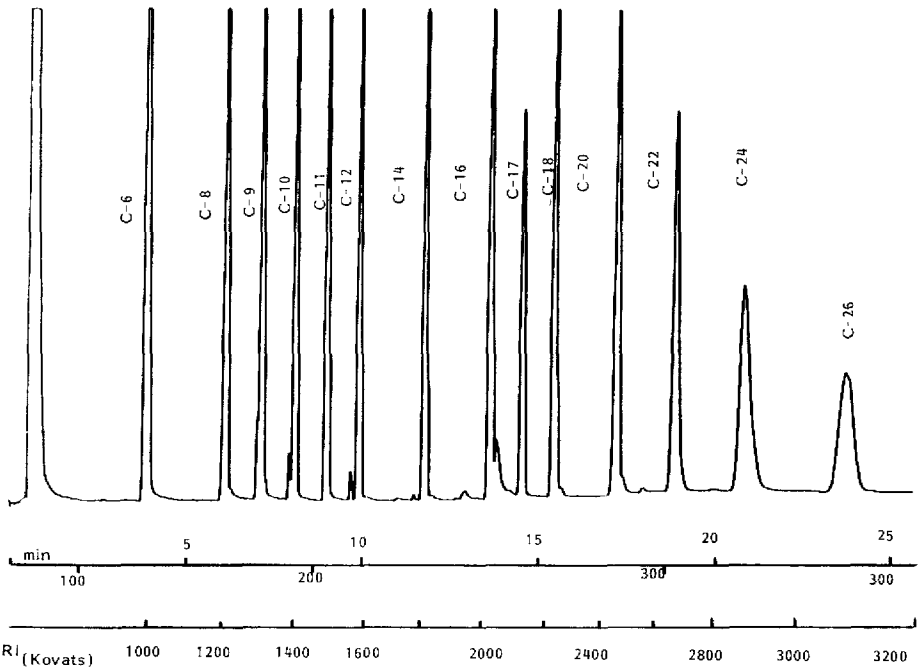

Fig. 2. Chromatogram of a mixture of 1-nitroalkanes (C_6–C_{26}) analyzed on a CP-Sil 5 column with thermionic detection. Temperature programme (°C) as in text. The lower scale shows the location of the *n*-alkanes.

TABLE II

KOVÁTS RETENTION INDICES OF DRUGS, MEASURED RELATIVE TO *n*-ALKANES AND CALCULATED FROM THE 1-NITROALKANE SCALE

CP-Sil 5 column. The reference values, on a packed OV-1 or SE-30 column[8], are given in parentheses.

Drug (RI ref.)	RI_{NO_2}	RI_{Kovats}	
		Calculated	*Measured*
Nicotine (1348)	887	1336	1332
Warfarin (1432)	949	1399	1395
Carbromal (1513)	1068	1520	1509
Demeton-S-methyl (1628)	1221	1676	1667
Lidocane (1870)	1412	1871	1881
Cyclobarbital(1963)	1498	1959	1967
Endosulfan α (2085)	1660	2124	2135
Endosulfan β (2175)	1760	2226	2236
Codeine (2376)	1935	2403	2391
Diazepam (2425)	1978	2448	2436
Diamorphine (2614)	2171	2644	2635
Fenetylline (2830)	2348	2825	2824

where y is the drug RI relative to n-alkane, x is the drug RI relative to 1-nitroalkane and r is the correlation coefficient. Thus it is possible to calculate the RI values from one scale by use of the other. This was checked by the determination of Kováts RI values of selected substances directly, using n-alkanes, and by the indirect method using RI of 1-nitroalkanes and the regression equation 3. The results, given in Table II, show good agreement between both sets of data. The homologues synthesize cover the whole range of toxicologically relevant substances, analyzed on an OV-1-like column (RI on n-alkane scale between 1000 and 3100).

CONCLUSIONS

1-Nitroalkanes are readily detectable with all GC detection systems used in modern toxicological analysis. Their chromatographic properties and close relation to n-alkanes allows the reliable calculation of retention index value from the Kováts n-alkane scale using our 1-nitroalkane scale and *vice versa*. This makes possible the convenient use of existing retention index libraries based on n-alkanes, for 1-nitroalkane work. No detector change is required. Apart from this immediate application, the 1-nitroalkanes may be a real alternative to the n-alkane scale in GC and HPLC.

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