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Note

Correction of retention index values in high-performance liquid chromatography as a tool for comparison of results obtained with different octadecyl silica phases

MACIEJ BOGUSZ*

Department of Toxicology, Institute of Forensic Medicine, Copernicus Academy of Medicine, Krakow (Poland)

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High-performance liquid chromatography (HPLC) is potentially a very valuable method for systematic toxicological screening, *i.e.*, for the identification of unknown substances in biological material. This results in the possibility use of two identification parameters, such as the retention behaviour and UV spectrum, in one analysis. The potential is, however, severely limited by poor interlaboratory reproducibility of results. The use of the retention time, relative retention time or capacity factor as a measure of retention behaviour was highly unsuccessful in terms of interlaboratory comparison; these parameters are strongly dependent on variations in the mobile phase composition, temperature, method of dead-time determination and in nominally identical stationary phases. Recently, Gill *et al.*[1] published the results of an interlaboratory study dealing with the assessment of various measures of retention behaviour, using exactly the same packing material. The best reproducibility was obtained with corrected capacity factors. Other measures of retention, like retention time, relative adjusted retention time and retention index, were less satisfactory.

The introduction of retention index (I) scaling in HPLC has significantly improved the reproducibility of retention behaviour[2–5]. Unfortunately, Smith *et al.*[6] observed that the I values for barbiturates, obtained by the use of different ODS-silica columns, showed large differences. Therefore these authors recommend the use of the same phase and possibly of the same batch when one wants to achieve highly reproducible results in an interlaboratory comparison. Such an approach, feasible perhaps on a regional level, makes practically impossible the broad use of collections of I values developed by other authors, *e.g.*, the use of I libraries in gas chromatography[7,8].

Similar difficulties were encountered years ago in regard to the interlaboratory comparison of R_F values in thin-layer chromatography (TLC), obtained from different chromatoplates. The problem was solved by the correction of R_F values, using appropriates standards analyzed along with the examined material[9–11]. The correction

* Present address: Institute of Forensic Medicine, Ruprecht-Karl University, Vossstrasse 2, D-6900 Heidelberg, F.R.G.

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of TLC data is routinely used in identification procedures in toxicology[12–15] and enables the use of comprehensive libraries of R_F values[15].

The purpose of this paper is to introduce the correction of I data on drugs, analyzed by HPLC using ODS-silica columns from different manufacturers. The principle of the correction was first used in TLC[9,12].

MATERIALS AND METHODS

A mixture of alkyl aryl ketones, acetophenone (50 mg/l), propiophenone (100 mg/l) and butyrophenone (200 mg/l) in methanol–water (1:1), was used as a reference mixture for I determination as described by Smith[3].

The solutions of barbiturates, barbital (200 mg/l), phenobarbital (200 mg/l), cyclobarbital (200 mg/l), butobarbital (400 mg/l), talbutal (400 mg/l), amobarbital (600 mg/l), pentobarbital (1000 mg/l), secobarbital (1000 mg/l) and methohexital (1000 mg/l), were prepared in methanol–water (1:1) with addition of sodium nitrate (5000 mg/l) as a dead-time marker.

The chromatographic separations were carried out with a Liquochrom 1010 Chromatograph (Labor MIM, Budapest, Hungary) equipped with a 5-μl sample loop and an UV detector set at 240 nm. Five commercially obtained columns were used: ODS-Si-Polyol 3 μm, 12.5 cm × 4.6 mm (Serva, Heidelberg, F.R.G.), ODS-Si-Polyol 5 μm, 25 cm × 4.6 mm (Serva): ODS Spherisorb 5 μm, 25 cm × 4.6 mm (Phase Separations; supplied by Pye Unicam, Cambridge, U.K.); ODS Hypersil 5 μm, 15 cm × 4.6 mm (Chrompack BV, Middelburg, The Netherlands); ODS Partisil 10 μm, 25 cm × 4.6 mm (Whatman; supplied by Pye Unicam). Methanol–phosphate buffer (40:60) pH 8.5[5] was used as a mobile phase at flow-rate of 1 ml/min. Each drug was analyzed in duplicate, and the retention indices were calculated from the k' values relative to the series of alkyl aryl ketones according to ref. 3.

In the last step, the I values were corrected using the method of Galanos and Kapoulas[9] which has been applied to toxicological identification[12,14]. The equations used were

$$I^c = aI + b \tag{1}$$

$$a = \frac{I_2^0 - I_1^0}{I_2 - I_1} \tag{2}$$

$$b = I_2^0 - aI_2 \tag{3}$$

where I_1^0 and I_2^0 are the listed values for the standards and I_1 and I_2 are the observed values for the standards. Using three correction standards (located in the low, middle and high I range; I_1, I_2 and I_3, respectively), three ranges were found: between t_0 ($I = 0$) and I_1, between I_1 and I_2 and between I_2 and I_3. The values located in particular ranges were corrected by application of appropriate standards and eqns. 1–3.

The data of Smith *et al.* (ref. 5, Table 1) on Hypersil ODS were used as a reference data base, and the I values for phenobarbital, talbutal and methohexital, listed in this paper, were chosen as correction standards.

TABLE I

UNCORRECTED AND CORRECTED *I* VALUES OF BARBITURATES FOUND ON FIVE DIFFERENT ODS-SILICA COLUMNS

Columns: 1 = Serva 3 μm; 2 = Serva 5 μm; 3 = Spherisorb 5 μm; 4 = Partisil 10 μm; 5 = Hypersil 5 μm.

*Drug**		*Column*				
		1	*2*	*3*	*4*	*5*
Barbital	u	572	556	520	534	582
(579)	c	604	602	606	587	590
Cyclobarbital	u	728	716	678	708	–
(762)	c	759	760	762	761	–
Butobarbital	u	762	750	712	742	786
(792)	c	791	789	792	791	792
Amobarbital	u	846	832	796	830	871
(875)	c	870	868	879	878	875
Pentobarbital	u	858	852	811	850	886
(890)	c	881	887	891	894	889
Secobarbital	u	906	892	854	890	927
(930)	c	928	926	950	933	929
Phenobarbital (676)		640	624	580	615	666
Talbutal (835)		810	798	760	790	830
Methohexital (1001)		980	970	896	960	1001

* u = Uncorrected, c = corrected.

TABLE II

PRECISION AND ACCURACY OF UNCORRECTED AND CORRECTED *I* VALUES OF BARBITURATES FOUND ON FIVE DIFFERENT ODS-SILICA COLUMNS

I and I^c = arithmetic mean ± S.D. for uncorrected and corrected *I* values, respectively, calculated on all five columns; d*I* and dI^c = mean difference ± S.D. between the uncorrected and corrected *I* values and listed *I* values, taken from ref. 5. The listed values are shown in parentheses.

Drug	*I*	*dI*	I^c	dI^c
Barbital (579)	553 ± 26	−26 ± 26	598 ± 9	19 ± 9
Cyclobarbital (762)	707 ± 21	−54 ± 21	760 ± 1	0.5 ± 1
Butobarbital (792)	750 ± 27	−42 ± 27	791 ± 1	−1 ± 1
Amobarbital (875)	835 ± 27	−40 ± 27	874 ± 5	−1 ± 5
Pentobarbital (890)	851 ± 27	−42 ± 22	890 ± 6	−0.4 ± 6
Secobarbital (930)	894 ± 27	−36 ± 27	933 ± 10	3 ± 10

Apart from the experimental investigations, the I values for barbiturates published by Smith *et al.*[6] for six different ODS-silica columns were recalculated according to the same correction principle.

RESULTS

Table I presents the uncorrected, I, and corrected, I^c, values for six barbiturates examined on five different ODS-silica columns. The I values for drugs show large variations and large deviations from the reference values. However, when the correction was applied, both the scatter of the results and the differences from the listed values diminished dramatically. Table II shows the precision (expressed as a standard deviation of the mean) and the accuracy (expressed as a difference between the experimental and listed value) of I and I^c values on all five columns. It is clear that the correction procedure improves the two parameters in question.

Table III shows the effect of the recalculation of the original data published

TABLE III

THE CORRECTION OF I VALUES FOR BARBITURATES FOUND ON SIX DIFFERENT ODS-SILICA COLUMNS BY SMITH *et al.*[6]

Columns: 1 = Hypersil 3 μm; 2 = Hypersil 5 μm; 3 = Techsil 5 μm; 4 = Spherisorb 5 μm; 5 = Zorbax 5 μm; 6 = Partisil 10 μm.

*Drug**		*Column*					
		1	*2*	*3*	*4*	*5*	*6*
Barbital	u	589	587	545	511	492	483
(579)	c	601	599	600	609	599	629
Cyclobarbital	u	752	751	705	667	663	631
(762)	c	763	760	765	762	774	763
Butobarbital	u	781	780	735	704	685	675
(792)	c	791	789	794	794	791	797
Heptabarbital	u	829	827	780	745	731	708
(838)	c	840	836	838	831	835	823
Amobarbital	u	865	864	817	786	769	759
(875)	c	875	872	873	873	869	873
Pentobarbital	u	882	879	835	807	790	789
(890)	c	892	887	891	893	888	901
Secobarbital	u	921	918	874	844	829	821
(930)	c	931	926	928	928	923	932
Phenobarbital (676)		662	660	614	567	555	517
Talbutal (835)		826	823	778	747	731	720
Methohexital (1001)		994	990	950	921	917	895

* u = Uncorrected values as in ref. 6; c = corrected values. Data listed in ref. 5 are shown in parentheses.

TABLE IV

PRECISION AND ACCURACY OF UNCORRECTED *I* VALUES FOUND FOR BARBITURATES ON SIX DIFFERENT ODS-SILICA COLUMNS BY SMITH *et al.*[6] AND THE EFFECT OF CORRECTION

I and I^c = arithmetic mean ± S.D. for uncorrected and corrected *I* values, respectively, calculated on all six columns. d*I* and dI^c = mean difference ± S.D. between the uncorrected and corrected *I* values and listed *I* values, taken from ref. 5. The listed values are shown in parentheses.

Drug	*I*	*dI*	I^c	dI^c
Barbital (579)	535 ± 47	−44 ± 47	606 ± 12	27 ± 12
Cyclobarbital (762)	693 ± 51	−69 ± 51	764 ± 5	3 ± 5
Butobarbital (792)	727 ± 46	−65 ± 46	793 ± 3	1 ± 6
Heptabarbital (838)	770 ± 51	−68 ± 51	834 ± 6	−4 ± 6
Amobarbital (875)	810 ± 47	−65 ± 47	872 ± 2	−2 ± 2
Pentobarbital (890)	830 ± 42	−60 ± 42	892 ± 5	2 ± 5
Secobarbital	868 ± 44	−62 ± 44	928 ± 3	−2 ± 3

by Smith *et al.*[6] (Table 4) for six different ODS-silica columns. Again, the data were corrected using the values from the ref. 5 and a striking improvement both in the precision and accuracy of results was observed (Table IV). Only in the case of barbital was there a marked difference between the listed *I* value and the I^c value; however, it is possible that the listed value in this case may be questionable. In ref. 5 the listed value for barbital, found at ambient temperature, is 579 ± 2 *I* units, whereas in the same paper the *I* value found at 20°C is 603 units. The latter value is close to the corrected ones (598 ± 9 in the present study and 606 ± 12 for the recalculated data from ref. 6). The temperature during the present study was ambient in the range 20–22°C.

DISCUSSION

The applied method of correction of *I* values in HPLC appeared very useful in the case of a rather uniform group of substances (barbiturates), analyzed on different ODS-silica columns. It may be assumed that the retention behaviour of chemically similar substances on different ODS-silica columns is highly correlated, as in the case of barbiturates. Therefore, the method applied may enable the comparison of retention data for chemically related substances obtained on different ODS-silica columns in different laboratories. The usefulness of the method for broader application, *e.g.*, for the identification of drugs belong to different chemical groups, needs further studies, which are in progress.

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