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Comparison of retention index scales based on alkyl aryl ketones, alkan-2-ones and 1-nitroalkanes for polar drugs on reversed-phase high-performance liquid chromatography

ROGER M. SMITH* and NOEL FINN
Department of Chemistry, Loughborough University of Technology, Loughborough, Leics. LE11 3TU (U.K.)
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ABSTRACT

The potential of alkan-2-ones, alkyl aryl ketones and 1-nitroalkanes as retention index standards in reversed-phase liquid chromatography has been examined using methanol–buffer, acetonitrile–buffer and tetrahydrofuran–buffer eluents. The aliphatic standards had retention times more similar to those of rapidly eluted polar drugs than the aromatic ketones. However, the retention indices of the drugs were sensitive to eluent composition and pH and to the brand of ODS-silica column material.

INTRODUCTION

Over the last ten years there have been a number of studies into the application of retention indices in high-performance liquid chromatography (HPLC) [1]. The two most widely applied homologous series of standards are the alkyl aryl ketones [2] and the alkan-2-ones [3] and both have been employed for the identification of drug compounds. They were selected because of their ready availability, reasonable stability and detectability using spectrophotometric detectors. Subsequently Bogusz and Aderjan have suggested the use of the homologous 1-nitroalkanes as retention index standards for HPLC [4] and gas–liquid chromatography [5].

All three series of standards are usable across a wide range of eluent composition but although the alkyl aryl ketones have the advantage of a strong chromophore, because of their larger size and thus longer retentions they cannot be readily applied to rapidly eluted polar drugs.

Bogusz [6] also examined the use of corrected retention indices to compensate for differences in the separations of the barbiturates on different makes of ODS-bonded stationary phases. Bogusz and co-workers subsequently reported the correction of retention indices of acidic [7], basic [8] and neutral [7,8] drugs on a range of ODS-silica stationary phases. In more recent work this approach has been extended to a comparison of the 1-nitroalkane and alkyl aryl ketone scales for corrected retention indices of basic drugs and they concluded that the former scale was superior [9].

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However, the two scales were compared using different test compounds and a re-evaluation of the results has found that the differences between the scales were negligible [10].

A major role of retention indices in chromatography is to increase the robustness of analysis results because as relative retention measurements they should compensate for small differences in the experimental conditions caused by variations between laboratories, instruments, or even in the preparation of the eluent on a different day or by a different operator. They can also be used as a reference scale to monitor differences between column and separation conditions, for interlaboratory comparisons and to establish databases of retention values.

The present study examines homologous alkyl aryl ketones, alkan-2-ones and 1-nitroalkanes as potential retention index standards and compares their retentions with those of a number of typical polar drug compounds in different acetonitrile–buffer, methanol–buffer and tetrahydrofuran (THF)–buffer eluents. Limited comparisons were also carried out of the effect of pH changes and the use of a different ODS-silica column.

EXPERIMENTAL

Materials and chemicals

The retention index standard compounds, nitromethane to nitrohexane, acetone to heptan-2-one and nonan-2-one were supplied by Aldrich (Poole, U.K.). The alkyl aryl ketones, acetophenone to valerophenone, column test compounds and drug test compounds were obtained from a number of sources. Methanol, acetonitrile, THF and water were HPLC grade and disodium hydrogen orthophosphate and potassium dihydrogen phosphate were analytical grade from FSA (Loughborough, U.K.).

Solutions

Standard solutions of nitroalkanes and alkyl aryl ketones (20–30 μl/500 ml) and alkan-2-ones (100–600 μl/5 ml) were prepared in mobile phase.

Buffer pH 7.0 was prepared from disodium hydrogenorthophosphate (0.50 g) and potassium dihydrogenphosphate (0.301 g) in water (1 l).

Equipment

HPLC separations were carried out using a Philips 4010 pump and Philips 4025 variable-wavelength detector set at 220 nm. The samples (10 μl) were injected using a 7125 Rheodyne valve, fitted with a 20-μl loop, onto a 100 × 5 mm column packed with either ODS-Hypersil (5 μm, batch 10/1229, Shandon Southern, Runcorn, U.K.), or ODS-Zorbax (7 μm, DuPont, Wilmington, DE, U.S.A.). The columns were thermostated at 30°C in a circulating water jacket.

The mobile phase was pumped at 1 ml/min and the column void volumes were detected using a solution of sodium nitrate (6.25 mg/ml). Retention times were determined using a Linseis chart recorder.

Procedure

The retention times were measured in triplicate and were used to calculate capacity factors. The retention indices (I) were calculated using $I = 100\ n + 100$

$(\log k'_x - \log k'_{R1})/(\log k'_{R2} - \log k'_{R1})$ where k'_{R1} and k'_{R2} are the capacity factors of the homologous retention index standards, containing n and $n + 1$ carbon atoms respectively, being eluted immediately before and after the analyte with retention k'_x. Some analytes were eluted outside the set of retention index standards and their indices were calculated by extrapolation from the closest available standards.

TABLE I

CAPACITY FACTORS OF RETENTION INDEX STANDARDS AND TEST COMPOUNDS

Conditions: column, ODS-Hypersil; eluent, modifier–phosphate buffer pH 7.0; detection, 220 nm.

Compound	Capacity factors									
	Methanol (%)				Acetonitrile (%)			THF (%)		
	20	30	40	50	10	20	30	20	30	40
Alkan-2-ones										
Acetone	0.88	0.63	0.54	0.45	0.98	0.72	0.53	0.55	0.40	–
Butan-2-one	1.97	1.38	1.01	0.74	2.33	1.54	1.12	0.83	0.75	0.64
Pentan-2-one	5.72	3.32	2.14	1.38	6.94	3.70	2.12	1.95	1.43	1.03
Hexan-2-one	18.21	9.30	5.13	2.81	22.16	10.19	4.85	5.13	2.96	1.79
Heptan-2-one	49.08	22.87	12.35	5.66	68.56	24.94	–	13.75	6.08	2.84
Nonan-2-one	–	149.03	53.26	19.06	–	131.92	–	99.87	21.92	6.68
1-Nitroalkanes										
Nitromethane	0.62	0.51	0.49	0.40	0.87	0.90	0.87	0.90	0.85	0.70
Nitroethane	1.32	1.07	0.86	0.65	2.28	1.99	1.53	1.60	1.44	1.06
Nitropropane	3.84	2.59	1.82	1.22	7.01	4.88	3.18	3.70	2.72	1.70
Nitrobutane	11.95	7.07	4.20	2.41	23.32	13.20	6.80	9.63	5.50	2.84
Nitropentane	38.66	19.83	10.28	5.11	81.92	37.50	15.10	25.80	11.05	4.53
Nitrohexane	129.72	59.09	26.20	11.08	–	108.40	34.18	70.10	21.54	7.10
Alkyl aryl ketones										
Acetophenone	21.94	10.35	5.29	2.63	31.92	11.14	4.87	6.46	3.18	1.64
Propiophenone	62.96	26.95	11.92	5.45	98.70	30.20	11.00	16.60	6.50	2.92
Butyrophenone	182.77	69.05	27.29	10.97	–	77.24	23.31	39.73	12.20	4.56
Valerophenone	–	195.17	67.07	–	–	–	–	100.90	23.13	7.08
Column test compounds										
N-Methylaniline	14.61	8.11	4.70	2.60	25.53	12.00	6.09	11.75	5.94	2.97
2-Phenylethanol	15.58	8.01	4.33	2.31	19.65	6.18	2.50	4.80	2.40	1.28
p-Cresol	19.03	9.92	5.19	2.65	29.11	10.10	4.09	15.40	5.94	2.55
Nitrobenzene	20.51	11.55	6.49	3.47	37.90	18.40	8.20	17.20	7.05	2.98
Toluene	80.01	45.86	24.55	12.14	–	60.99	23.43	54.07	17.72	6.42
Drug compounds										
Aspirin	0.97	0.46	0.24	0.11	1.00	0.11	0.06	0.06	0.04	–
Paracetamol	1.58	0.84	0.57	0.41	1.92	0.81	0.51	0.94	0.67	0.48
Theophylline	2.34	1.11	0.64	0.40	1.93	0.61	0.33	0.52	0.32	0.24
Barbitone	4.62	2.20	1.18	0.69	5.61	1.66	0.78	2.45	1.70	1.13
Salicylamide	5.66	2.85	1.49	0.88	8.42	2.77	1.37	4.17	1.99	1.09
Caffeine	4.66	1.88	1.01	0.64	4.61	0.95	0.54	0.53	0.36	0.27
Phenobarbitone	13.48	5.34	2.22	1.06	24.36	4.96	1.62	8.14	3.76	1.91
Phenacetin	23.85	9.28	4.25	2.11	34.40	–	–	4.55	1.85	1.01
Diazepam	–	–	–	–	–	–	–	37.82	7.30	2.52

RESULTS AND DISCUSSION

In order to study the relative retentions of the standards and drug compounds and to determine the influence of eluent composition on retention indices, the capacity factors of members of three homologous series, the alkan-2-ones (acetone to nonan-2-one), the nitroalkanes (nitromethane to nitrohexane) and the alkyl aryl ketones (acetophenone to valerophenone) were measured using a range of buffered pH 7.0 eluents, containing methanol, acetonitrile or THF (Table I). The retentions were also measured for a selection of polar neutral, basic and acidic drug compounds and for a set of aromatic column test compounds (nitrobenzene, toluene, *p*-cresol, 2-phenylethanol and N-methylaniline). These last compounds had been chosen in a previous study as characterising the different interactions between eluent and stationary phases [11]. All the compounds gave good peak shapes and reproducible results.

One reason for selecting a homologous series of standard compounds to form a retention index scale is that they are usually systematically spaced throughout the chromatogram. In an isocratic separation there is normally a linear relationship between the logarithm of the capacity factor (log k') and the carbon number of each homologue. The differences in log k' between successive homologues are the methylene increments, which in a particular eluent should be constant and independent of the functional groups present. However, differences have been found in earlier work between homologous alkyl aryl ketones and alkan-2-ones [2] and between derivatives of homologous alcohols and amines [12].

However, although the correlation coefficients were good for a linear relationship between carbon number and log k' for the alkyl aryl ketones (0.9999–0.9995 except for 40% THF 0.9976), the correlations for the alkan-2-ones and nitroalkanes were generally much poorer (0.9995–0.9943) and the relationships were curved (Figs. 1 and 2). Nitromethane and nitroethane appeared to be more highly retained than would be anticipated. Similar deviations from linearity were also noted for nitromethane by Bogusz and Aderjan [4] in acetonitrile–water eluents. The methylene increments for

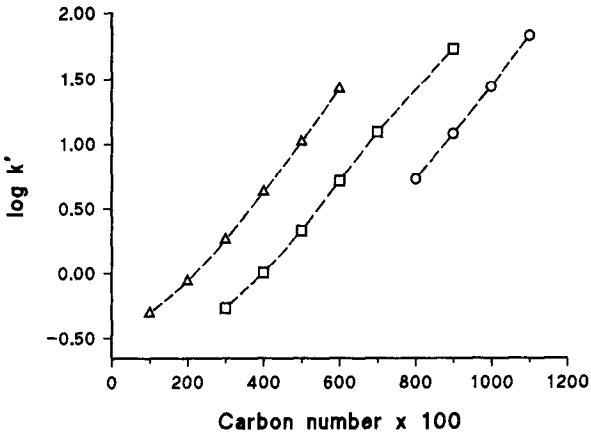

Fig. 1. Relationship of log k' to carbon number × 100 for the three homologous series alkyl aryl ketones (○), alkan-2-ones (□) and nitroalkanes (△). Eluent, methanol–buffer pH 7.0 (40:60).

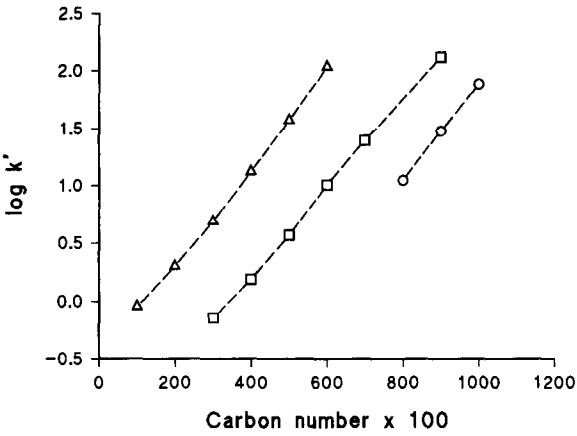

Fig. 2. As Fig. 1. Eluent, acetonitrile–buffer pH 7.0 (20:80).

each set of standards were different and generally increased with the carbon number although a drop was noted on going from heptan-2-one to nonan-2-one.

The retention indices using each of the set of homologues were therefore determined by interpolation between the logarithms of the capacity factors of adjacent pairs of standards. The indices based on the alkyl aryl ketones [I(AAK)], nitroalkanes [$I(NO_2)$] and alkan-2-ones [I(CO)] were compared in a typical eluent [Table II, methanol–buffer (40:60)]. The retention index of acetophenone, the smallest alkyl aryl ketone (C_8) [I(CO) = 619, $I(NO_2)$ = 426] is close to that of hexan-2-one and a little larger than the value for nitrobutane. Acetone [$I(NO_2)$ = 118] and nitromethane [$I(NO_2)$ = 100] the smallest members of the other two series had very similar retentions. The alkyl aryl ketones were more highly retained than the drug compounds and extrapolation of the scale was therefore required to determine the I(AAK) values. In contrast all the drugs, with the exception of aspirin, fell within the ranges of the two sets of aliphatic standards demonstrating the increased application of these scales for rapidly eluted compounds.

In order to compare the effects of the eluent composition on the relative retentions, the retention indices I(CO) of all the analytes in the different eluents were calculated based on the alkan-2-one scale. On increasing the proportion of methanol the indices of the nitroalkanes increased only slightly but those of the alkyl aryl ketones decreased (Table III). With the exception of toluene, whose retention index increased noticeably with the proportion of methanol, the retention indices of the column test compounds also changed very little. This confirmed the robustness of the retention indices of non-ionised compounds towards small changes in eluent composition. Similar but larger effects were observed on increasing the proportion of acetonitrile or THF (Table III). The differences between the retention indices measured using the different modifiers are a measure of the changes in the elution selectivities of the eluents.

However, in all three eluent combinations the retention indices for most of the polar drugs decreased significantly, by up to 150 units, with increased proportions of

TABLE II

COMPARISON OF RETENTION INDICES CALCULATED USING DIFFERENT SCALES IN METHANOL–BUFFER (40:60)

Conditions: column, ODS-Hypersil; eluent, methanol–phosphate buffer pH 7.0 (40:60); spectroscopic detection, 220 nm. Indices in parentheses have been calculated by extrapolation.

Compound	Retention index scale		
	I(CO)	I(NO_2)	I(AAK)
Alkan-2-ones			
Acetone	300[a]	118	(520)
Butan-2-one	400	219	(596)
Pentan-2-one	500	319	(689)
Hexan-2-one	600	422	(796)
Heptan-2-one	700	520	904
Nonan-2-one	900	(676)	1076
1-Nitroalkanes			
Nitromethane	(257)	100[a]	(507)
Nitroethane	342	200	(577)
Nitropropane	426	300	(669)
Nitrobutane	564	400	(772)
Nitropentane	676	500	882
Nitrohexane	798	600	992
Alkyl aryl ketones			
Acetophenone	619	426	800[a]
Propiophenone	(723)	516	900
Butyrophenone	(833)	(607)	1000
Valerophenone	–	(700)	1100
Column test compounds			
N-Methylaniline	589	412	785
2-Phenylethanol	582	404	777
p-Cresol	603	425	799
Nitrobenzene	626	448	825
Toluene	794	593	985
Drug compounds			
Aspirin	(170)	(<0)	(420)
Paracetamol	308	124	(526)
Theophylline	327	147	(540)
Barbitone	421	242	(616)
Salicylamide	452	273	(644)
Caffeine	400	221	(596)
Phenobarbitone	504	324	(693)
Phenacetin	579	401	(774)

[a] Index standards I = carbon number × 100.

modifier (Table III, Fig. 3). These effects were expected from previous studies of the changes in the contributions of different functional groups to retention [13], which found that more polar groupings, such as amido, amino, hydroxyl and phenolic groups, showed larger changes with composition than did the relatively non-polar nitro and carbonyl groups. Thus the retention of compounds which are rapidly eluted

TABLE III

RETENTION INDICES BASED ON ALKAN-2-ONES OF RETENTION INDEX STANDARDS AND TEST COMPOUNDS

Conditions as in Table I. Retention indices in parentheses are derived by extrapolation from the standards.

Compound	Retention index I(CO)									
	Methanol (%)				Acetonitrile (%)			THF (%)		
	20	30	40	50	10	20	30	20	30	40
1-Nitroalkanes										
Nitromethane	(257)	(273)	(284)	(277)	(286)	330	367	409	419	419
Nitroethane	342	367	374	374	398	429	449	477	501	504
Nitropropane	426	472	478	480	501	527	549	566	590	590
Nitrobutane	564	573	577	578	604	629	(640)	664	686	700
Nitropentane	676	684	679	685	(716)	750	(737)	764	793	809
Nitrohexane	798	801	803	811	–	876	(836)	864	897	(914)
Alkyl aryl ketones										
Acetophenone	619	612	604	591	631	610	600	623	610	584
Propiophenone	(723)	717	694	691	732	723	(697)	719	710	706
Butyrophenone	(833)	818	812	809	–	836	(789)	807	809	811
Valerophenone	–	(929)	931	–	–	–	–	(901)	(908)	(913)
Column test compounds										
N-Methylaniline	581	587	589	589	612	618	(620)	684	697	710
2-Phenylethanol	586	586	582	572	589	550	520	593	571	539
p-Cresol	604	608	603	592	624	599	579	712	697	676
Nitrobenzene	612	624	626	630	647	666	(663)	723	723	711
Toluene	(749)	774	794	828	–	807	(790)	838	867	890
Drug compounds										
Aspirin	312	(260)	(170)	(44)	302	(54)	(8)	<0	<0	–
Paracetamol	373	337	308	(282)	378	315	(295)	415	379	(340)
Theophylline	416	372	327	(277)	378	(278)	(236)	(287)	(240)	(194)
Barbitone	481	454	421	386	480	408	352	524	524	517
Salicylamide	499	483	452	428	517	467	432	579	546	510
Caffeine	485	435	400	371	463	336	303	(292)	(262)	(219)
Phenobarbitone	574	546	504	458	608	529	(602)	647	633	614
Phenacetin	627	600	579	559	639	529	–	588	535	496
Diazepam	–	–	–	–	–	–	–	802	727	674

because they contain a number of polar functions are likely to alter to a different extent on changing the composition of the eluent than smaller less polar compounds, which are rapidly eluted because of their small size. Thus it appears that the application of these retention index scales to very polar analytes may be inherently limited because the values will be susceptible to small changes in eluent composition. Because the relative retentions of the nitroalkanes and alkan-2-ones were largely unchanged with composition the effects on indices based on either scale would be similar.

Because of concern that the nitroalkanes could be ionised in high-pH eluents the capacity factors and retention indices of the standards and test compounds were measured at pH 3.2 and pH 8.2 using methanol–buffer (50:50) and acetonitrile–buffer

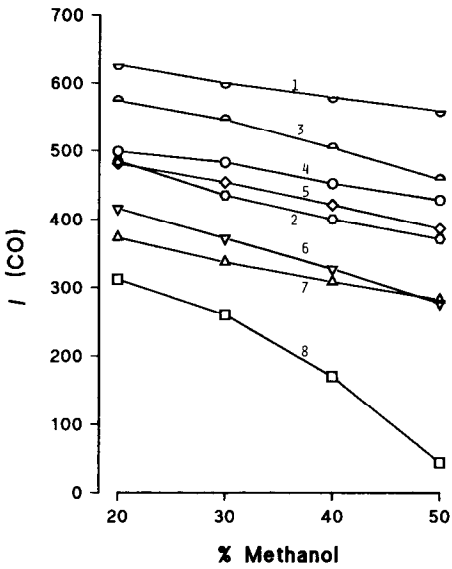

Fig. 3. Effect of proportion of methanol on the retention indices I(CO) of drug compounds on ODS-Hypersil column. Compounds: 1 = phenacetin; 2 = caffeine; 3 = phenobarbitone; 4 = salicylamide; 5 = barbitone; 6 = theophylline; 7 = paracetamol; 8 = aspirin.

(30:70) eluents. However, no significant changes were observed in the relative retentions of the three homologous series or the neutral column test compounds. Because of protonation N-methylaniline was more rapidly eluted at pH 3.2 and its retention index dropped by 70 units in methanol and 113 units in acetonitrile compared to pH 7.0. The retention indices of the acidic drugs, salicylamide, barbitone and phenobarbitone all decreased at pH 8.2, by up to 140 units, reflecting partial ionisation.

Different stationary phases

In order to determine if the relationships between the three retention index scales were similar on different brands of ODS-silica the separations were repeated on an ODS-Zorbax column using methanol–buffer pH 7.0 (50:50), and the capacity factors and retention indices [I(CO)] were calculated (Table IV). Except for nitroethane the differences for the nitroalkanes were less than 15 units but the alkyl aryl ketones were relatively more highly retained, by up to 40 units, on the ODS-Zorbax column.

Larger differences were found for the column test compounds, with 2-phenylethanol (−68 units) and *p*-cresol (−87 units) being more rapidly eluted and nitrobenzene (+47 units) and toluene (+50 units) being more retained on the ODS-Zorbax column. These selectivity changes match those observed earlier for polar compounds on an ODS-Zorbax column, which has a generally lower retention for compounds containing hydroxylic or phenolic groups [11].

These marked differences between the selectivity of the column materials were also reflected in the retention of the acidic drugs paracetamol, barbitone, salicylamide,

TABLE IV

COMPARISON OF CAPACITY FACTORS AND RETENTION INDICES ON DIFFERENT STATIONARY PHASES

Conditions: eluent, methanol–buffer pH 7.0 (50:50). H = Hypersil; Z = Zorbax.

Compound	k'		I(CO)	
	ODS-H	ODS-Z	ODS-H	ODS-Z
Alkan-2-ones				
Acetone	0.45	0.51	300	300
Butan-2-one	0.74	0.80	400	400
Pentan-2-one	1.38	1.23	500	500
Hexan-2-one	2.81	2.32	600	600
Heptan-2-one	5.66	4.07	700	700
Nonan-2-one	19.06	15.73	900	900
1-Nitroalkanes				
Nitromethane	0.40	0.44	(277)	(267)
Nitroethane	0.65	0.63	374	347
Nitropropane	1.22	1.14	480	482
Nitrobutane	2.41	2.00	578	578
Nitropentane	5.11	3.89	685	692
Nitrohexane	11.08	7.71	811	794
Alkyl aryl ketones				
Acetophenone	2.63	2.81	591	634
Propiophenone	5.45	5.11	691	733
Butyrophenone	10.97	9.13	809	819
Valerophenone	–	18.07	–	920
Column test compounds				
N-Methylaniline	2.60	2.29	589	598
2-Phenylethanol	2.31	1.26	572	504
p-Cresol	2.65	1.27	592	505
Nitrobenzene	3.47	3.53	630	675
Toluene	12.14	13.60	828	878
Drug compounds				
Paracetamol	0.41	0.31	(282)	(189)
Theophylline	0.40	0.44	(277)	(267)
Barbitone	0.69	0.40	386	(245)
Salicylamide	0.88	0.57	428	326
Caffeine	0.64	1.50	371	533
Phenobarbitone	1.06	0.44	458	(267)
Phenacetin	2.11	1.27	559	503

phenobarbitone and phenacetin, which all had lower relative retentions on the ODS-Zorbax column. In contrast, caffeine was more highly retained.

In his work on corrected retention indices Bogusz [6] demonstrated that it was possible to compensate for differences in the retention indices of barbiturates on ODS-Hypersil and ODS-Zorbax columns. His technique corrected for systematic differences in the retentions of the analytes and the retention index scale standards by scaling the indices relative to those found on a "standard" column. However, this

approach will fail in the present study as the order of elution is different on the two different ODS-phases. These conditions are likely to occur in other cases when there are major differences in the test and standard stationary phases. Analytes which contain different interactive groups may be eluted in a different order. For these columns the correction method will only work reliably for closely related groups of compounds with a "pseudo-homologous" relationship (such as the barbiturates) in which all the analytes have the same functional groups and similar polarity but differ in the extent of alkyl substitution.

CONCLUSION

Both the alkan-2-one and 1-nitroalkane homologues had similar retention times to a range of rapidly eluted basic drugs and thus should be more suitable as the basis of retention scales for these compounds than the more highly retained alkyl aryl ketones. However, at short retention times the relative retentions of the standards and drugs are susceptible to small changes in the proportion of modifier in the eluent. The retention indices can therefore only partially compensate for small changes in the separation conditions and this emphasises the importance of controlling the separation parameters in qualitative analysis.

Large differences in selectivity were found between two different ODS-bonded silica columns which suggests that attempts to correct retentions on "equivalent" ODS-bonded silica stationary phases may have limited applicability. For the reliable comparison of results in different laboratories it will still be necessary to closely specify the brand of stationary phase.

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