Z Rechtsmed (1987) 98:263–268

Zeitschrift für
Rechtsmedizin

Instrumental Set-up as a Factor Influencing the Variability of Retention Index Values in High Pressure Liquid Chromatography

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Summary. The influence of different laboratory set-ups on HPLC retention index values of selected acidic and basic drugs was investigated. The RI values of drugs were calculated using the alkyrarylketone scale. Two columns filled with different batches of the same Hypersil ODS phase were consecutively used in two laboratories under identical conditions, but using different instrumentation. The study showed that the different laboratory set-ups influence the precision of RI values, and the differences in column filling affect the accuracy.

Key words: HPLC, retention indices – Retention index values, HPLC

Zusammenfassung. Es wird über den Einfluß unterschiedlicher instrumenteller Bedingungen in zwei verschiedenen Laboratorien auf die in der HPLC mittels Alkylarylketonen berechneten Retentionsindices ausgewählte Arzneistoffe berichtet: Zwei Säulen wurden mit verschiedenen Chargen Hypersil ODS Phase gefüllt und jeweils nacheinander in zwei Laboratorien unter identischen Bedingungen aber verschiedener Instrumentenausstattung eingesetzt. Die Untersuchung zeigt, daß das Säulenfüllungsmaterial die Richtigkeit und die apparative Komponente die Genauigkeit der Retentionsindex-Werte beeinflußt.

Schlüsselwörter: HPLC, Retentionsindices – Retentionsindex-Werte, HPLC

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Introduction

The introduction of a retention index system in high pressure liquid chromatography (HPLC) has significantly diminished the intra- and interlaboratory variability of results in comparison with such measures of retention as relative retention time or capacity factor [1–3]. Nevertheless, factors, such as differences in nominally identical stationary phase, slight differences in mobile phase composition or temperature may still contribute to variability in retention index values [1, 4].

The purpose of this study was to examine the influence of different laboratory set-ups on the retention index values of chosen acidic and basic drugs, using the same columns installed in sequence in two laboratories.

Material and Methods

Two columns, filled with different batches of the same Hypersil ODS 5 phase (Shandon Southern, UK), were used. One batch was supplied by the Drug & Toxicology Division, Central Research Establishment, Home Office Forensic Science Service, Aldermaston, UK (batch no. 10/1229), and the second batch was obtained commercially (batch no. 728). This particular phase was chosen because British authors had published retention data for barbiturates using the same Hypersil ODS phase, batch 10/1229. This allowed us to compare the results with the two columns in the present study and also with the results obtained by Smith et al. [4, 5]. The columns in this study (15 cm long, 4.6 mm ID) were packed in Groningen (The Netherlands) under identical conditions and consecutively used in two laboratories – in Groningen and Heidelberg (FRG). Table 1 shows the drugs used for the experiments and the chromatographic conditions. The sequence of investigations was as follows: First, the series of experiments with basic drugs was performed in Groningen. Afterward, the same columns

Table 1. Materials and methods

Acidic drugs (μg/10 μl injected)	Basic drugs (μg/10 μl injected)
Amobarbital (5)	Caffeine (2)
Aprobarbital (2)	Chlordiazepoxide (1)
Barbital (1)	Codeine (2)
Butobarbital (2)	Methaqualone (2)
Methohexital (10)	Strychnine (4)
Pentobarbital (4)	
Phenobarbital (1)	
Secobarbital (5)	
Talbutal (5)	

Mobile phases:

Acidic drugs:	Methanol-phosphate buffer according to [7]. Detection at 240 nm. Flow 2 ml/min
Basic drugs:	Methanol-water (75:25) containing 0.05% ammonia (min. 25%). Detection at 254 nm. Flow 2 ml/min

were installed in Heidelberg, where the study for acidic and basic drugs was performed. Then the columns were sent back to Groningen where the study for acidic drugs was completed.

The following instrumentation was used during the study: In Groningen: A Spectra Physics Pump Type 4800 connected with a Waters Intelligent Sample Processor WISP and Kratos UV Detector Type 757. In Heidelberg: A Type 414 Pump, Series 2000 Programmer, MSI 660 Sampler and Uvikon 725 Detector – all from Kontron AG.

All chromatographic conditions were kept identical. The temperature of analysis was ambient in the range 20 to 23°C.

The study on acidic drugs was based on the collaborative trial, organized by the Central Research Establishment in the UK and published recently [6]. The measurements were performed in series during 5 consecutive days for each column in Heidelberg and during 4 consecutive days in Groningen. Each day a new portion of mobile phase was prepared. The retention index values were calculated against alkylarylketones according to Smith [7], and the dead time was determined with sodium nitrate. The effective plate number was monitored daily with butyrophenone. The series of chosen basic drugs were analyzed 5-fold during 5 consecutive days in both laboratories. The retention index values were also calculated against the series of alkylarylketones using sodium nitrate as a dead time marker.

Results

The scheme applied in the study enabled the forming of four clusters of data, which reflect the intralaboratory variability of RI values for a given batch of stationary phase. However, it is also possible to make combinations of data, e.g., for the assessment of intralaboratory variability of results obtained with two different columns or for the assessment of interlaboratory variability of results obtained with the same column. The total variability may be calculated from all data together (Table 2).

Table 3 shows the retention index values for barbiturates obtained during the study. The absolute differences between the laboratories did not exceed 6 RI units. Also, the results were very close to the values published by Smith et al. [4, 5]. The intralaboratory variability of results in Heidelberg was distinctly higher than in Groningen, both for individual columns and for combination. The variability did not depend on the column filling. The analysis of variance showed that the laboratory and the batch of stationary phase did not exert a significant influence on the mean RI values of the drugs (Table 4).

Table 2. Possible combinations of results

Lab. 1/Col. 1	Lab. 1/Col. 2	Lab. 2/Col. 1	Lab. 2/Col. 2
Lab. 1/two columns		Lab. 2/two columns	
Intralaboratory variability			
Col. 1/two labs		Col. 2/two labs	
Interlaboratory variability			
Two labs/two columns			
Overall variability			

Table 3. Day-to-day variability of RI values for barbiturates (mean ± RSD %)

	Groningen		Heidelberg	
	Col. 728	Col. 10/1229	Col. 728	Col. 10/1229
Barbital	582 ± 0.2	588 ± 0.9	590 ± 1.8	586 ± 2.1
Phenobarbital	666 ± 0.4	668 ± 0.2	663 ± 1.7	660 ± 0.6
Aprobarbital	734 ± 0.2	737 ± 0.1	746 ± 1.9	735 ± 0.4
Butobarbital	786 − 0.2	788 − 0.2	783 − 1.3	785 − 0.4
Talbutal	830 − 0.2	832 − 0.2	827 − 1.0	828 − 0.4
Amobarbital	871 − 0.1	872 − 0.2	867 − 1.0	867 − 0.4
Pentobarbital	886 − 0.4	887 − 0.2	882 − 1.5	885 − 0.4
Secobarbital	927 − 0.1	929 − 0.2		
Methohexital	1001 − 0.3	1000 − 0.1	996 − 0.9	994 − 0.4
	Groningen (two columns)	Heidelberg (two columns)	Col. 728 (two labs)	Col. 10/1229 (two labs)
Barbital	585 − 0.8	588 − 1.9	586 − 1.4	586 − 1.6
Phenobarbital	667 − 0.5	661 − 1.4	664 − 1.4	663 − 0.8
Aprobarbital	735 − 0.2	740 − 1.5	741 − 1.6	739 − 1.4
Butobarbital	787 − 0.3	784 − 0.9	784 − 1.0	786 − 0.4
Talbutal	831 − 0.2	827 − 0.8	828 − 0.9	829 − 0.5
Amobarbital	871 − 0.3	867 − 0.7	869 − 0.8	870 − 0.5
Pentobarbital	886 − 0.3	884 − 1.0	884 − 1.1	886 − 0.2
Secobarbital	928 − 0.2	992 − 0.7		
Methohexital	1001 − 0.2	995 − 0.7	998 − 0.7	996 − 0.4
	Two labs/ two columns			
Barbital	587 − 1.5			
Phenobarbital	664 − 1.1			
Aprobarbital	738 − 1.2			
Butobarbital	785 − 0.7			
Talbutal	828 − 0.7			
Amobarbital	869 − 0.6			
Pentobarbital	885 − 0.8			
Methohexital	998 − 0.6			

Table 5 presents the results obtained for basic drugs. The average variability of results was higher here than in the case of barbiturates, most probably due to the kind of mobile phase, which was more prone to changes in the composition (ammonia). Also in this case the variability was greater in Heidelberg, for each column alone and for the combination.

The analysis of variance showed that the RI values of basic drugs were column-dependent, i.e., dependent on the batch of stationary phase, and the 2-way interaction of column and laboratory was also significant. This means that the kind of stationary phase and the laboratory both influence the RI values (Table 6).

Table 4. The analysis of variance of results obtained for barbiturates

	DF	*F*	*P*
Main effects			
Substances	7	5003	<0.001
Laboratories	1	0.3	0.599
Columns	1	0.7	0.409
2-Way interactions			
Subst./labs	7	1.6	0.149
Subst./columns	7	1.9	0.071
Labs/columns	1	0.4	0.505
Residual	119		

Table 5. Day-to-day variability of RI values for basic drugs (mean − RSD %)

	Groningen		Heidelberg	
	Col. 728	Col. 10/1229	Col. 728	Col. 10/1229
Caffeine	627 − 0.4	652 − 0.7	661 − 4.6	621 − 2.7
Codeine	836 − 1.1	837 − 0.4	865 − 2.5	822 − 1.4
Methaqualone	875 − 0.9	882 − 0.3	899 − 1.6	875 − 1.4
Chlordiazepoxide	946 − 0.7	950 − 0.2	966 − 1.2	950 − 1.2
Strychnine	1051 − 1.4	976 − 1.0	1072 − 1.9	965 − 0.7
	Groningen (two columns)	Heidelberg (two columns)	Col. 728 (two labs)	Col. 10/1229 (two labs)
Caffeine	638 − 2.2	643 − 4.8	645 − 4.4	636 − 2.7
Codeine	836 − 0.7	843 − 3.0	851 − 2.3	830 − 1.3
Methaqualone	882 − 0.3	887 − 1.8	890 − 1.4	879 − 0.9
Strychnine	1015 − 4.6	1024 − 5.7	1069 − 1.2	971 − 1.0
	Two labs/ two columns			
Caffeine	641 − 3.7			
Codeine	840 − 2.2			
Methaqualone	884 − 1.3			
Chlordiazepoxide	954 − 1.1			
Strychnine	1020 − 5.1			

Discussion

The present study showed that different laboratory set-ups may influence the precision of the retention index values for acidic and basic drugs. The impact of laboratory equipment involved on the variability (i.e., the precision) was larger than the influence of the difference in batch number of the same stationary phase. Furthermore, the mean RI-values (i.e., accuracy) were dependent on

Table 6. The analysis of variance of results obtained for basic drugs

	DF	*F*	*P*
Main effects			
Substances	3	2169	<0.001
Laboratories	1	2.13	0.15
Columns	1	21.5	0.001
2-Way interactions			
Subst./labs	3	0.42	0.742
Subst./columns	3	1.18	0.323
Labs/columns	1	47.8	<0.001
Residual	59		

the column used, i.e., on the batch of stationary phase. The latter was especially pronounced for basic drugs.

This confirms the finding of Smith et al. [4] who recommended the use of the same batch of stationary phase for interlaboratory use. This condition is, however, almost impossible to fulfil in practice. The systematic differences between the laboratories, caused both by differences in stationary phases or laboratory set-up, may interfere in the interlaboratory use of reference databases. These interferences, however, may be overcomed by correcting the retention data, as was shown by Gill et al. for k′ values [6], and by Bogusz for RI-values [8].

Acknowledgment. The authors express their thanks to Dr. R. Gill from the Central Research Establishment, Home Office Forensic Science Service, Aldermaston, UK, for kindly supplying the Hypersil ODS phase batch 10/1229 and the protocol of the barbiturate study.

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Received April 2, 1987