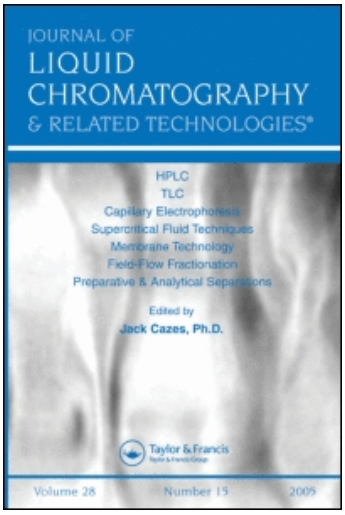

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COMPARABILITY OF RP-HPLC RETENTION INDICES OF DRUGS IN THREE DATABASES

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ABSTRACT

Three databases of HPLC retention indices in the 1-nitroalkane scale were recently established (IFM= Institute of Forensic Medicine, MCL = Microchemistry Laboratory, MTSS= Merck Tox Screening System). In two of them (IFM and MTSS) virtually identical chromatographic conditions were applied but different RP-columns. In the third system (MCL) a different column and different elution conditions were used. Among a total of 1149 drugs examined in all three labs, 258 drugs were common for IFM and MTSS, 179 were common for MCL and MTSS, 162 were common for IFM and MCL, and 135 drugs were common for all three databases.

Good agreement of retention index values in IFM and MTSS was demonstrated (r^2 = 0.9778). The correlations between IFM and MCL and MTSS and MCL data were less strong (r^2 = 0.9245 and 0.9339, respectively), due to some deviating results. These deviations concerned mainly substances with pKa values between 1 - 4 and were probably caused by the differences in the pH of the two mobile phases. The standardization of chromatographic conditions is a prerequisite of interlaboratory reproducibility of retention indices.

INTRODUCTION

An unequivocal identification of toxic substances in biological samples in the case of acute poisoning is of critical importance in clinical and forensic toxicology. The multitude of potentially toxic substances in the human environment dictates a need for such methods of identification, which allow for the examination of the largest possible number of relevant compounds in one analytical run. For this reason, chromatography is a method of choice in general toxicological screening.

Among various chromatographic techniques, thin layer chromatography (TLC) and gas chromatography (GC) have found broad application for identification of toxic substances. Both techniques have been standardized and respective databases, comprising retention parameters for over 6.000 toxicologically relevant compounds have been established.[1-3] The application of hyphenated techniques like e.g. GC/MS[4] or TLC/UV[5] have resulted in particularly high identification power.

High pressure liquid chromatography (HPLC) may be regarded as a very attractive alternative in systematic toxicological screening. This method is more sensitive and selective than TLC, and more universal than GC, enabling direct examination of non-volatile, polar and thermolabile drugs. The standardization of HPLC results with retention index (RI) scales made it possible to establish databases of relevant compounds. Among the numerous retention index systems tested in HPLC, three have been widely applied. They are the alkan-2-ones, alkyl aryl ketones and 1-nitroalkanes.[6,7] The latter scale[8] has been proven useful for identification of various acidic, neutral and basic drugs in reverse phase HPLC and was applied for general toxicological screening.[9] It was observed, that the differences in selectivities of various brands of reverse phase packings may result in very different RI values of the same substances when analyzed on commercially different HPLC columns. This problem was circumvented to some

extent by the application of selected drugs as secondary retention index standards.[10] However, the elution conditions should be carefully standardized in order to obtain reproducible results.[11-12]

The HPLC identification system was developed under standardized conditions, using the 1-nitroalkane retention index system, selected drugs as secondary RI standards and a diode array detector (DAD). In this system a database of 225 compounds was established.[13] This database was expanded to about 400 toxicologically relevant drugs and endogenous compounds at the Institute of Forensic Medicine in Aachen (IFM).[14] The RI values were not affected by co-extracted biological matrices.[15] The overall identification power of combined HPLC-RI and UV data was comparable with the identification power of combined GC retention indices and UV spectral data.[16]

Recently, Hill and Kind from the Microchemistry Laboratory in Storrs, CT (MCL) published a RI library comprising data for 469 drugs.[12] Also, the RI library of Merck Tox Screening System (MTSS) with data for some 900 substances has become commercially available.[17] In both databases the 1-nitroalkane RI scale was used. The existence of these three libraries, containing a number of common substances, made it possible to assess the ruggedness of the HPLC retention index system based on the 1-nitroalkane scale.

Two out of three libraries (IFM and MTSS) were developed in virtually identical elution conditions, but different columns were used. This made it possible to compare the RI-values of drugs examined under standardized conditions. On the other hand, in the third lab (MCL) a different column and different gradient elution conditions were applied. This could help to examine, to what extent the RI-values of drugs may be affected by these differences, and to identify particularly sensitive compounds.

An ultimate purpose was to inspect the possibility of the interlaboratory use and exchange of retention data.

MATERIALS AND METHODS

Among a total of 1149 drugs examined, the following number of common drugs was observed:

258 drugs were common for IFM and MTSS databases,
179 drugs were common for MCL and MTSS databases,
162 drugs were common for IFM and MCL databases.
135 drugs were examined in all three laboratories.

Table 1

HPLC Conditions Applied in Three Laboratories Establishing HPLC/RI Databases

Library	Column	Mobile Phase	Gradient Profile	Flow Rate
IFM (n = 404)	Superspher 100 RPI8EC 125 x 4 mm	A:TEAP buffer pH 3.0 B: CH_3CN	0 to 70% B in 30 min 5 min 70% B pH 3.0 - 4.2	1 mL/min temp. ambient*
MCL (n= 469)	Zorbax RXC_8 250 x 4.6 mm	A:0.15M H_3PO_4 0.05M TEA in H_2O B: 0.15M H_3PO_4 0.05M TEA 20% H_2O in CH_3CN	2.2 min 100% A 0 to 100% B in 30 min 5 min 100%B overall pH 2.2	2 mL/min temp. 30 °C
MTSS (n=875)	Lichrospher 60 RP Select B 125 x 4 mm	A: TEAP buffer pH 3.0 B: CH_3N	0 to 70% B in 30 min 5 min 70%B pH 3.0 - 4.2	1 mL/min temp. 25 °C

The RI values of common drugs were subjected to comparison directly or after correction, using the method described elsewhere.[9,12,13] Separate secondary RI standards were used for acidic drugs (paracetamol, barbital, brallobarbital, pentobarbital, secobarbital, clobazam, indomethacine) and for basic drugs (morphine, chloroquine, benzoylecgonine, cocaine, diphenhydramine, haloperidol, amitriptyline, thioridazine and meclozine). Only the RI-values of drugs eluting inside the elution range of secondary standards were corrected.

The primary and secondary RI data were subsequently subjected to comparative analysis. Table 1 shows the HPLC conditions applied in three labs developing RI libraries.

RESULTS AND DISCUSSION

All applied HPLC systems (IFM, MTSS and MCL) assured good selectivity for acidic, neutral and basic drugs throughout the whole elution range (Fig.1). All three columns exhibited low base absorptivity due to base-deactivation (Superspher or Lichrospher Select B) or the use of type B silica (Zorbax RX). It was demonstrated by several authors, that these column packings show some silanol activity which may be effectively suppressed by addition of an

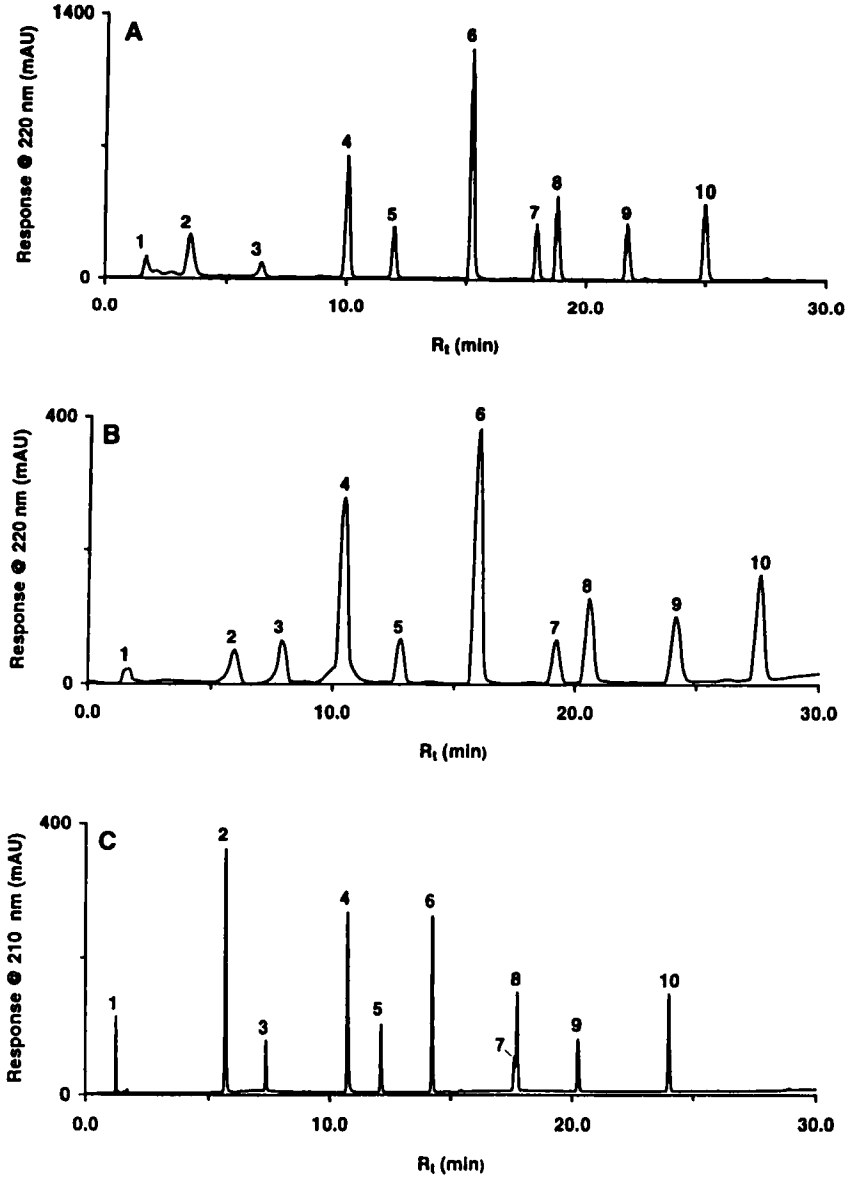


Figure 1: Chromatograms of representative drugs analyzed on the (A) MTSS, (B) IFM and (C) MCL HPLC systems. (1) nicotine, (2) morphine, (3) atenolol, (4) caffeine, (5) primidone, (6) noscapine, (7) pentobarbital, (8) imipramine, (9) thioridazine, (10) indomethacin.

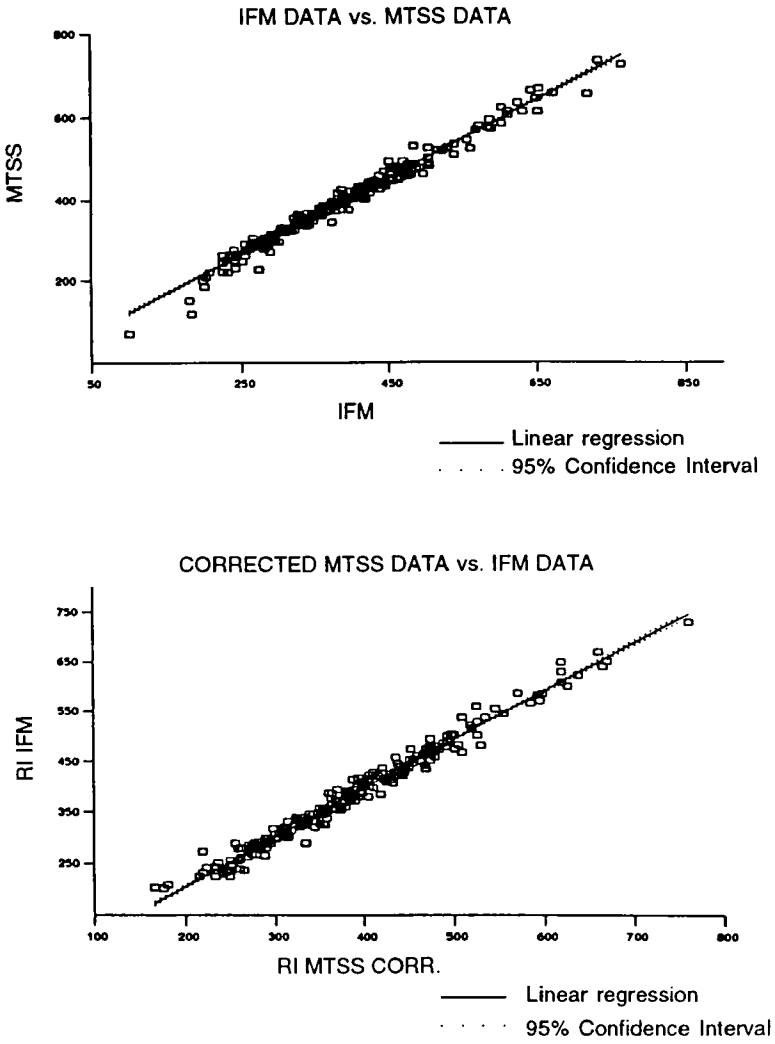


Figure 2: Comparison of RI-values (uncorrected and corrected data) for common drugs in IFM and MTSS databases.

amine to the mobile phase.[18-21] The gradient elution the in acidic mobile phase with addition of an amine modifier was proved superior to isocratic procedures and has been applied for screening purposes in toxicology.[12,13,15,22,23]

Table 2

Retention Indices, Available pKa Values and Scale Applied for Correction of Data in Three Databases

	Substance	MCL	IFM	MTSS	Scale	Pk_a
1	Acebutolol		311	325	BAS	91.4
2	Acecarbromal		422	429	ACN	0.0
3	Acepromazine	385	399		BAS	9.3
4	Acetanilide	328	316		ACN	0.6
5	Acetophenazine	345		395	BAS	
6	Allobarbital		340	346	ACN	7.8
7	Alprazolam		443	470	ACN	2.4
8	Amiloride		233	257	BAS	8.7
9	Aminophenazone		243	262	ACN	5.0
10	Amiodarone		762	731	BAS	5.6
11	Amitriptyline	430	446	440	BAS	9.4
12	Amobarbital	410	415	404	ACN	7.9
13	Amoxapine	336	372	398	BAS	0.0
14	Amphetamine	242	241	244	BAS	9.9
15	Apomorphine	282		348	BAS	
16	Aprobarbital	355	347	357	ACN	8.0
17	Aspirin	348	326	350	ACN	3.5
18	Atenolol	235	224	243	BAS	9.6
19	Atropine	297	287	306	BAS	9.9
20	Barbital	298	287	308	ACN	8.0
21	Benperidol		371	393	BAS	
22	Benzocaine	374	380	404	BAS	2.8
23	Benzoic Acid	354		360	ACN	4.2
24	Benzoylecgonine	304	295	295	BAS	
25	Benztropine	444	461		BAS	10.0
26	Bisacodyl		483	531	ACN	
27	Brallobarbital	359	359	371	ACN	
28	Bromazepam	348	378	397	ACN	2.9
29	Bromisoval		354	365	ACN	10.8
30	Brompheniramine	279	355		BAS	3.9
31	Brucine	303	288	312	BAS	2.3
32	Buflomedil		324	347	BAS	
33	Bupivacine		355	366	BAS	8.1
34	Bupranolol		373	391	BAS	
35	Buprenorphine	384	386	397	BAS	8.5

(continued)

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
36	Buspirone	350	353	369	BAS	
37	Butacaine	366		392	BAS	
38	Butalbital	373	380	394	ACN	7.6
39	Butaperazine		496	464	BAS	
40	Butobarbital		365	384	ACN	8.0
41	Caffeine	295	265	305	BAS	1.0 14.0
42	Camazepam	549	566		ACN	
43	Carazolol		354	381	BAS	
44	Carbamazepine	399	380	418	BAS	
45	Carbromal	398	400	410	ACN	
46	Chloramphenicol	368		390	ACN	
47	Chlordiazepoxide	338	357	363	ACN	4.6
48	Chloroquine	273	265	282	BAS	8.4
49	Chlorotheophylline 8	300	295		BAS	
50	Chlorpromazine	438	466	456	BAS	9.3
51	Chlorprothixene		476	459	BAS	8.8
52	Chlorthalidone		359	367	ACN	9.4
53	Cimetidine	236	229	251	BAS	6.8
54	Cinchonidine	379		396	BAS	4.2 8.4
55	Clenbuterol	316	311	326	BAS	
56	Clobazan		484	488	ACN	
57	Clomethiazole		469	495	BAS	3.2
58	Cloimipramine		471	462	BAS	
59	Clonazepam	443	451	465	ACN	1.5
60	Clonidine		237	258	BAS	8.2
61	Clopamide		356	377	ACN	
62	Clopenthixol		456	448	BAS	
63	Chlorazepate	376	464	475	ACN	3.5
64	Clozapine		338	368	BAS	
65	Cocaine	327	336	348	BAS	8.6
66	Codeine	258	243	266	BAS	8.2
67	Colchicine	379	357	382	BAS	1.7
68	Coumarin	383	368		ACN	
69	Cyclobarbital		374	384	ACN	7.6
70	Cyclopentobarbital		374	391	ACN	
71	Demoxepam		388	416	ACN	4.5
72	Desipramine	414	421		BAS	10.2
73	Dextromethorphan	367	370	377	BAS	8.3

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
74	Dextromoramide		440	440	BAS	
75	Dextropropoxyphene	409	438	442	BAS	6.3
76	Diamorphine	328	327	340	BAS	7.6
77	Diazepam	458	529	528	ACN	3.3
78	Diazoxide		345	368	ACN	8.5
79	Dibenzepine		349	361	BAS	
80	Diclofenac	614	630	616	ACN	4.5
81	Digoxin	387	389		BAS	
82	Dihydrocodeine		237	261	BAS	8.8
83	Dilthiazen	393	392		BAS	
84	Dimethindene		334	338	BAS	
85	Dimethoxymethylamphe	353		371	BAS	
86	Diphenhydramine	372	385	393	BAS	9.0
87	Diprophylline	264		275	BAS	
88	Dipyridamole		387	393	BAS	6.4
89	Disopyramide		372	345	BAS	8.4
90	Disulfiram	731	730	741	ACN	
91	Dothiepine		428	428	BAS	
92	Doxepin	379	401	404	BAS	9.0
93	Droperidol		369	385	BAS	
94	Ephedrine	231	224	221	BAS	9.6
95	Estrazolam		424	445	ACN	
96	Estrone	554		544	ACN	
97	Ethacrynic Acid	564	516	521	ACN	3.5
98	Ethomidate		466	475	ACN	4.2
99	Ethonitazene	361		406	ACN	
100	Ethorphine	328		344	BAS	
101	Ethosuximide	297	284	301	ACN	9.5
102	Ethylephrine		183	118	BAS	9.0
103	Ethylmorphine	289	283	291	BAS	
104	Ethyltolilmalonamide	328		338	ACN	
105	Famotidine	228		233	BAS	6.7
106	Fenbufen		522	520	ACN	4.5
107	Fencamfamine	345		354	BAS	
108	Fenetylline		325	366	BAS	
109	Fenfluramine		364	371	BAS	9.1
110	Fenoprofen	565	587	574	ACN	4.5
111	Fentanyl	377	377	373	BAS	9.0

(continued)

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
112	Flecainide		410	419	BAS	
113	Fluanisone		398	423	BAS	
114	Flubendazole	392		454	BAS	
115	Flufenamic Acid	687		671	ACN	3.9
116	Flumazenil		362	387	ACN	
117	Flunarizine		571	581	BAS	
118	Flunitrazepam	480	459	483	ACN	1.8
119	Fluorouracil	135		70	ACN	8.0 13.0
120	Flupenthixol		487	475	BAS	
121	Fluphenazine	391	480	462	BAS	3.9
122	Flurazepam		392	397	ACN	1.9
123	Flurosemide	422	414	435	ACN	3.9
124	Glibenclamide		623	637	ACN	5.3
125	Gliclazide		538	536	ACN	5.8
126	Glipizide	493	464	478	ACN	0.0
127	Gluthetimide	437	430	436	ACN	9.2
128	Guaifenesine		307	328	ACN	0.0
129	Haloperidol	403	409	421	BAS	8.3
130	Harmane		301	319	BAS	
131	Heptabarbital		404	416	ACN	0.0
132	Hexobarbital	416	404	419	ACN	8.2
133	Hexylresorcinol	561		579	ACN	
134	Homatropine	260		272	BAS	9.7
135	Hydrochlorothiazide	289	275	294	ACN	7.0
136	Hydrocodone	268	262	286	BAS	8.3
137	Hydromorphone	234	231	220	BAS	8.2
138	Hydroxyzine		428	437	BAS	2.1
139	Ibuprofen	620	650	616	ACN	4.4
140	Imipramine	424	434	437	BAS	9.5
141	Indomethacin	612	610	607	ACN	4.5
142	Isoniazid	67	132		ACN	1.8
143	Ketamine	293	294	311	BAS	7.5
144	Ketazolam		583	577	BAS	
145	Ketoprofen	500	501	495	ACN	4.5
146	Ketotifene		365	373	BAS	
147	Labetalol		350	365	BAS	7.4
148	Levomepromazine		446	435	BAS	9.2
149	Levorphanol	303	308		BAS	8.2

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
150	Lidocaine	282	285	288	BAS	7.9
151	Lorazepam	423	422	444	ACN	1.3
152	Lormetazepam	484	474	487	ACN	
153	Loxapine	355	399	407	BAS	6.6
154	LSD	342	358	362	BAS	7.5
155	Lysergic-D Acid	285	269		ACN	3.4
156	Maprotiline		440	438	BAS	
157	Mazindol	347		357	ACN	
158	MDA	253	261	278	BAS	
159	MDE	291	244		BAS	
160	MDMA	274	280	278	BAS	
161	Mebendazole	382		438	BAS	
162	Meclozine	602	601	587	BAS	3.1
163	Medazepam	355	395	405	ACN	6.2
164	Mefenamic Acid	695	670	661	ACN	4.2
165	Melperone		327	360	BAS	
166	Mepacrine	355	331	345	BAS	7.7
167	Mephenesine	358	349	364	BAS	
168	Mephenytoin	395	382		ACN	8.1
169	Mepivacaine	288	300	296	BAS	7.7
170	Mescaline	258	255	272	BAS	
171	Mesuximide	422	458		ACN	
172	Metamizol		289	316	ACN	
173	Metapirylene	234		342	BAS	3.7 8.9
174	Methadon	435	443	440	BAS	8.3
175	Methamphetamine	259	255	262	BAS	10.0
176	Methaqualone	435	455	449	BAS	2.5
177	Methohexital		503	503	ACN	8.3
178	Methoxyamphetamine	263		274	BAS	
179	Methyl Phenidate	311	316	328	BAS	8.8
180	Methyldopamine	166	166		BAS	10.6 2.2 9.2 12.0
181	Methylphenobarbital		420	435	ACN	7.8
182	Methyltestosteron	619	580		ACN	
183	Methyprylon	338		347	ACN	
184	Metoclopramide		308	324	BAS	9.0
185	Metoprolol	313	317	326	BAS	9.7
186	Metronidazol		236	257	ACN	2.5

(continued)

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
187	Mianserine		390	391	BAS	
188	Midazolam		386	399	ACN	6.2
189	Mondacetylmorphine-6	274	276		BAS	
190	Moperon		387	406	BAS	
191	Morphine	212	198	200	BAS	8.0
192	Morphine-3-Glucur.	206	167		BAS	
193	Nadolol	274	271	288	BAS	
194	Nalorphine	243	237	260	BAS	7.8
195	Naloxone	258	251		BAS	7.9
196	Naproxene	492	476	488	BAS	4.2
197	Nefopam	355	344		BAS	9.2
198	Nicotine	64	100	69	BAS	3.2
199	Nifedipine	531	503	527	ACN	
200	Niflumic Acid	561	586	595	ACN	0.0
201	Nikethamide		280	304	ACN	3.5
202	Nimodipine		641	668	ACN	
203	Nitrofurantoin		307	319	ACN	7.2
204	Nitrazepam	404	430	448	ACN	3.2
205	Nitrendipine		601	625	ACN	
206	Nomifensine		346	349	BAS	
207	Nordiazepam	377	464	470	ACN	3.5
208	Nortriptyline	419	418	430	BAS	9.7
209	Noscapine	339	354	368	BAS	6.2
210	Opiramol		387	377	BAS	3.8
211	Orciprenaline		180	151	BAS	9.0
212	Orphenadrine		416	418	BAS	8.4
213	Oxazepam	422	441	441	ACN	1.7
214	Oxazolam		340	339	ACN	
215	Oxprenolol		332	354	BAS	9.5
216	Oxycodone	262	260	277	BAS	8.9
217	Oxymorphone	222		217	BAS	
218	Oxyphenbutazone	516	503	501	ACN	4.7
219	Oxytetracycline	278		299	ACN	7.3 9.1 3.3
220	Papaverine	335	346	363	BAS	6.4
221	Paracetamol	257	234	264	ACN	9.5
222	Paroxetin		385	426	BAS	
223	Pecazine		440	443	BAS	9.7
224	Pemoline	292	281	307	BAS	10.5

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
225	Penfluridol		716	659	BAS	
226	Pentazocine	358	357	372	BAS	8.5
227	Pentobarbital	409	405	424	ACN	8.0
228	Pentoxiphylline		320	355	BAS	
229	Perazine		418	403	BAS	
230	Periciazine		405	410	BAS	
231	Perphenazine		438	428	BAS	7.8
232	Pethidine		334	345	BAS	8.7
233	Phenacetin	371	356	377	ACN	2.2
234	Phenazocine	386		409	BAS	
235	Phenazone		303	333	ACN	1.5
236	Phencyclidine	355	356	375	BAS	8.5
237	Phenelzine	193	200	184	BAS	
238	Pheniramine	216	279	283	BAS	4.2
239	Phenobarbital	366	357	379	ACN	7.4
240	Phenothiazine	668		665	BAS	
241	Phenprocoumon		609	616	ACN	
242	Phentermine	269	277		BAS	10.1
243	Phenylbutazone	687	651	672	ACN	4.4
244	Phenylcyclohexylamin	329		323	BAS	
245	Phenylephrine	101		80	BAS	9.8 8.8
246	Phenytoin		415	431	ACN	8.3
247	Physostigmine	277	267	296	BAS	1.8
248	Pindolol		277	300	BAS	9.7
249	Pipamperone		286	299	BAS	
250	Piribedil		301	328	BAS	
251	Piritramid		395	377	BAS	
252	Piroxicam		425	431	BAS	
253	Prazepam	572	648	648	ACN	2.7
254	Primidone	320	308	322	ACN	
255	Probenecid	541	560	526	ACN	3.4
256	Procainamid		202	208	ACN	9.2
257	Procaine		236	264	BAS	9.0
258	Prochlorperazine		462	450	BAS	8.1
259	Progesteron	797		672	ACN	
260	Promazine		418	407	BAS	9.4
261	Promethazine		411	409	BAS	9.1
262	Propafenone		408	433	BAS	

(continued)

Table 2 (continued)

	Substance	MCL	IFM	MTSS	Scale	Pk_a
263	Propiomazine	394		440	BAS	6.6
264	Propranolol	351	370	377	BAS	9.5
265	Propyhenazone		422	441	ACN	
266	Protriptyline		424	418	BAS	
267	Quinidine	261	316	322	BAS	4.2
268	Quinine	276	308	327	BAS	4.1
269	Ranitidine	229	240		BAS	2.3
270	Reserpine	437	473	467	BAS	6.6
271	Resorcinol	348		243	ACN	6.2
272	Saccharin	295	268	291	BAS	1.6
273	Salbutamol	225	207	220	ACN	9.3
274	Salicylamide	319	305	327	ACN	8.2
275	Salicylic Acid	375	331	359	ACN	3.0
276	Scopolamine	256	288	270	BAS	7.6
277	Secbutabarbital		365	377	ACN	
278	Secobarbital	434	437	437	ACN	7.9
279	Sotalol		273	226	BAS	8.3
280	Spironolactone		504	502	ACN	
281	Strychnine	296	292	302	BAS	2.3
282	Sulfadiazine	271	260		ACN	6.5
283	Sulfanilamide	125	142		ACN	10.4
284	Sulpiride		240	250	BAS	8.9
285	Sulthiam		321	344	ACN	10.0
286	Temazepam	473	466	472	ACN	1.6
287	Terfenadine		567	571	BAS	
288	Testosterone	573		534	ACN	
289	Tetracaine	354	381	389	BAS	8.5
290	Tetracycline	292		314	ACN	7.7 9.5 3.3
291	Tetramizole	259		261	ACN	
292	Tetrazepam		538	511	ACN	
293	Thebaine	316	324	340	BAS	8.2
294	Theobromine	259	224	262	BAS	1.0 14.0
295	Theophylline	270	239	276	BAS	1.0 14.0
296	Thiabendazole	266	283		ACN	
297	Thiamylal	509		516	ACN	7.3
298	Thiopental		481	485	ACN	7.6
299	Thioridazine	454	504	490	BAS	9.5
300	Tiaprofenic Acid		475	484	ACN	3.0

Table 2 (continued)

Substance		MCL	IFM	MTSS	Scale	Pk_a
301	Timolol		297	317	BAS	
302	Tocainide		251	247	BAS	7.8
303	Tolazamide	479		452	ACN	5.7
304	Tolazoline	217		225	BAS	10.3
305	Tolbutamide	474	470	477	ACN	5.3
306	Tolmetine	468		470	BAS	3.5
307	Trancylpromine	232	241	230	BAS	8.2
308	Trazodone		258	278	BAS	
309	Triamcinolone	366		348	ACN	
310	Triamterene		291	298	BAS	6.2
311	Triazolam		452	476	ACN	
312	Trichlormethiazide	385	377		ACN	8.5
313	Trifluoperazine	396	491	480	BAS	8.1
314	Trifluperidol		436	459	BAS	
315	Triflupromazine		505	484	BAS	9.4
316	Trihexyphenidyl	416	410	429	BAS	
317	Trimethoprim	286	289	299	ACN	7.2
318	Trimethoxyamphetamin	269		290	BAS	
319	Trimipramin		451	454	BAS	
320	Tripelenamine	237	337	336	BAS	3.9
321	Tripolidine	276	360	388	BAS	6.5
322	Tropacocaine	319		332	BAS	9.7
323	Tryamine	109	124		BAS	9.5
324	Verapamil	437	454	447	BAS	
325	Viloxazine		321	325	BAS	
326	Vinylbital		410	424	ACN	
327	Warfarin	538	555	546	ACN	5.0
328	Yohimbine	318	333	340	BAS	
329	Zopiclone		314	331	BAS	

Table 2 shows the uncorrected RI values for all common drugs, as well as the available pKa values and the ionization character of each compound.

The comparison of IFM and MTSS data showed a high agreement of RI-values (Fig.2). More than 90% of all results showed deviations in the range of ± 20 RI units.

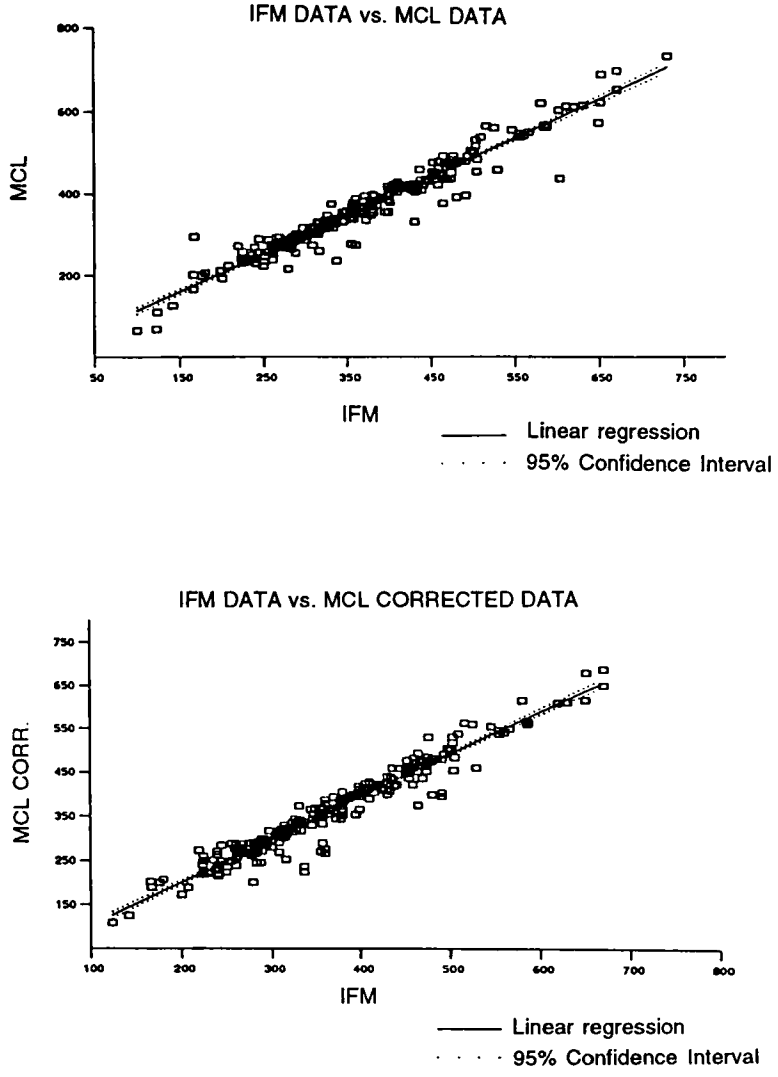
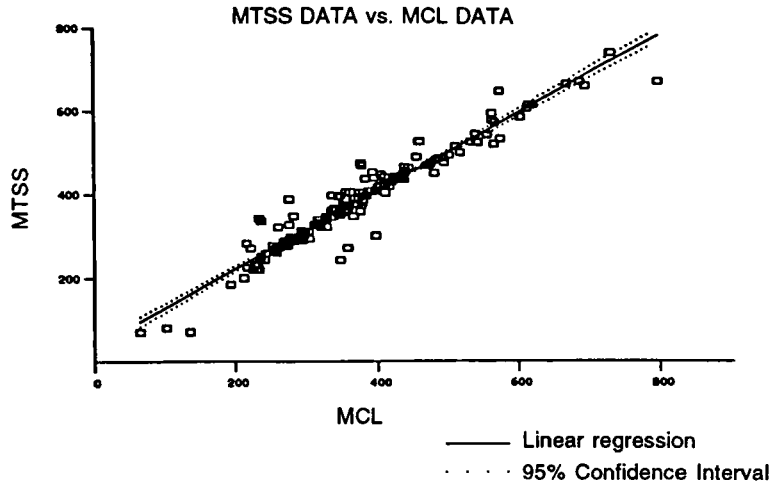


Figure 3. Comparison of RI-values (uncorrected and corrected data) for common drugs in IFM and MCL databases.



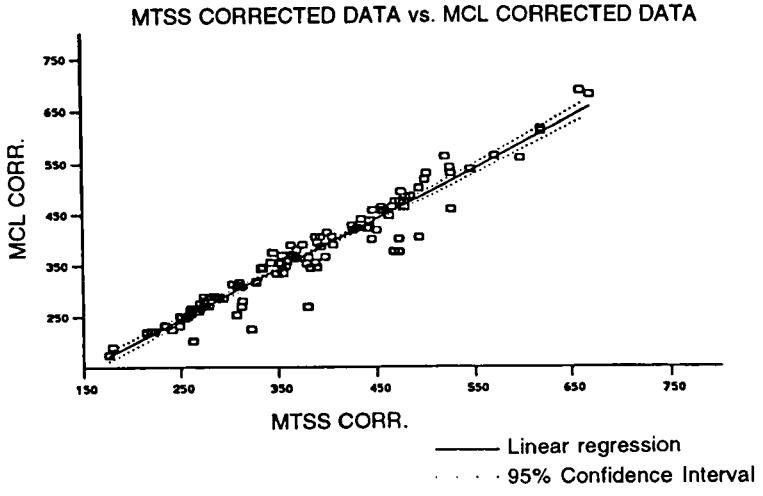


Figure 4. Comparison of RI-values (uncorrected and corrected data) for common drugs in MCL and MTSS databases.

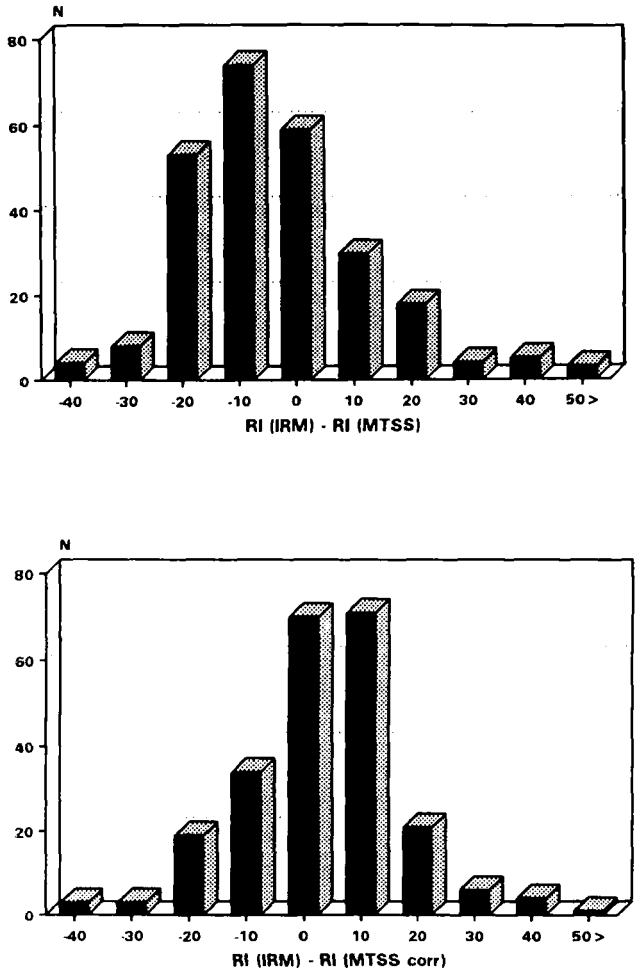


Figure 5. Differences in RI-values of common drugs (uncorrected and corrected data) for common drugs in IFM and MTSS databases

Retention indices obtained in MCL also showed good agreement with IFM and MTSS data in most of cases. However, several distinct deviations were observed, which have influenced the correlation (Figs.3,4). The correction procedure resulted in only a slight improvement of the correlation values. These results are similar to the comparative data published recently[12] (Table 3).

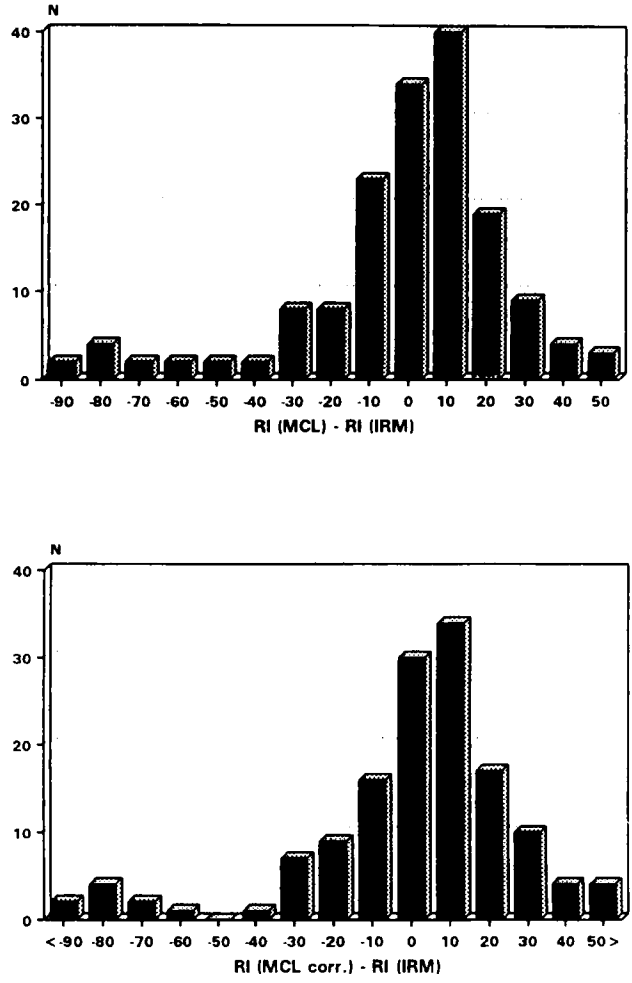


Figure 6. Differences in RI-values of common drugs (uncorrected and corrected data) for common drugs in IFM and MCL databases.

Figs. 5-7 show the distribution of differences in RI-values between particular databases. Considering these data, the search window of 30 RI units may be recommended when the retention data obtained are compared with a database

Table 3

Correlation Coefficients of RI-Values of Common Drugs Examined in Three Databases

Library	r^2 Value Uncorrected Data	r^3 Value Corrected Data
MCL/IFM*	0.9059	0.9132
IFM/MTSS	0.9778	0.9805
IFM/MCL	0.9245	0.9267
MCL/MTSS	0.9339	0.9368

* comparison of data published by Bogusz and Wu[12] with data of Hill and Kind[16] - 90 common drugs

established in standardized conditions in another laboratory. The search window in intralaboratory use of the user-created library practically never needed to exceed 10 RI units. The same experience was shared by the users of the MTSS system, who apply exactly the same conditions and the same column (Lichrosorb Select B, supplied together with the system) in various laboratories.

Evaluation of the data obtained in two laboratories using virtually the same conditions but different columns (IFM and MTSS) disclosed some groups of drugs showing particularly high variability, i.e.:

-xanthine derivatives (caffeine, theophylline, theobromine, fenetylline and pentoxiphylline),
-some benzodiazepine derivatives (alprazolam, demoxepam, triazolam, flunitrazepam),.
-pyrazolone derivatives (phenazone, metamizol),
-salicylates (salicylic acid, asperin).

A common feature of these compounds, as well as some others showing distinct differences in RI-values (physostigmine, brucine, ibuprofen, probenecid) was the presence of functional groups with low pKa values (1 - 4.5). It might be possible that the chromatographic mobilities of these drugs are more susceptible to the small differences in apparent pH values of the mobile phase due to the different degree of ionization. The analysis of RI-data obtained in MCL in comparison with data obtained in IFM or MTSS also showed some regularities.

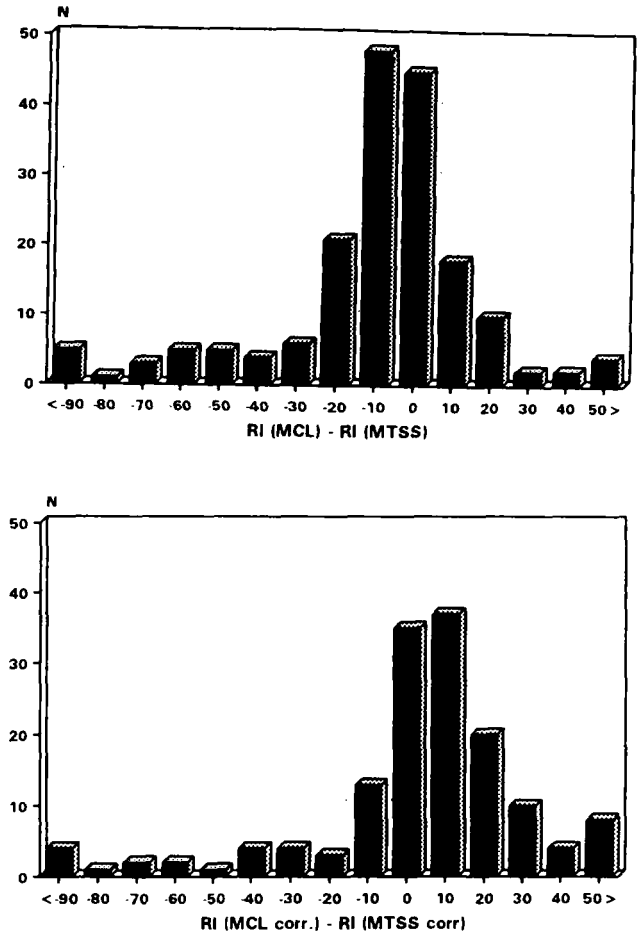


Figure 7. Differences in RI-values of common drugs (uncorrected and corrected data) for common drugs in MCL and MTSS databases

Drugs showing distinctly lower RI-values in the MCL library were some phenothiazine derivatives and basic drugs with pKa values below 4 (e.g., tripenelamine, fluphenazine, diazepam, nordiazepam, prazepam). Acidc drugs with low pKa values showed higher RI-values than in IFM or MTSS libraries. The comparison of HPLC conditions shows that the pH of the system used in the MCL was distinctly lower than in the IFM or MTSS. Moreover, the elution system applied in the MCL assured the same pH value of 2.2 throughout

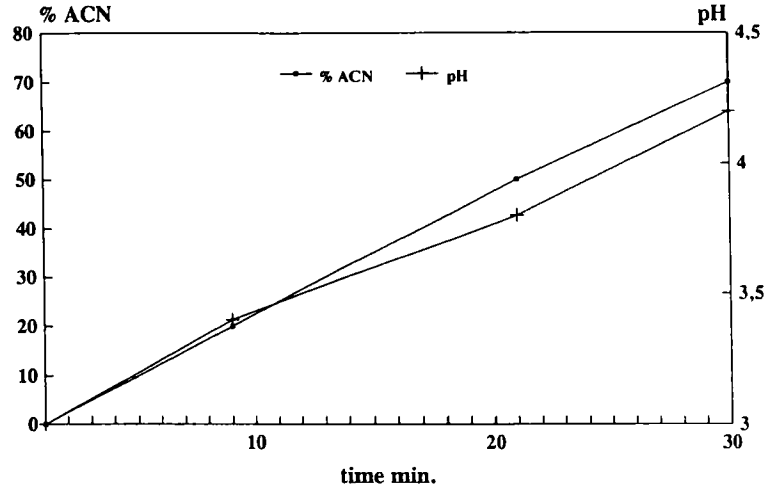


Figure 8. Changes in apparent pH of mobile phase during gradient elution in IFM/MTSS system.

the whole elution range. This was not the fact for the system used in the IFM and MTSS. Measurements of the pH of the mobile phase during elution showed a steady rise from pH 3.0 at the beginning to 4.2 at the end of gradient profile (Fig. 8). This means that the difference in pH of the mobile phase between MCL and IRM/MTSS systems increased with the time of analysis. The lower pH value of the mobile phase in the MCL system most probably caused higher ionization and higher mobility of basic compounds having pKa values in critical range[1-4] and an opposite effect on acidic drugs. This in consequence may have caused the differences in observed relative retention values (RI's).

The pKa values showed in Table 2 were taken from several sources.[24-30] Unfortunately, not all pKa were available to us, which makes the interpretation of some outlying data more difficult.

The structures of most of the compounds that we have analyzed indicate the presence of more than one ionizable functional groups. Moreover, it should be stressed that the pKa values listed in the literature are for the drugs in aqueous solutions. In the presence of organic solvents these values may be

substantially shifted. This was demonstrated for benzodiazepine derivatives, which have pKa values of the base ionizable group in acetonitrile 7 to 8 units higher in acetonitrile than in water,[31] or for diclofenac, showing linear increase in its pKa value from 4.0 to 6.8 with increasing concentrations of ethanol.[25]

Another factor that may affect the chromatographic mobility of some compounds is the possible difference in concentrations of metal ions in different stationary phases. This may cause different chelation of solutes and in consequence a shift in RI values. This could be the cause in the variable RI results obtained for salicylic acid.

The interaction of drugs with active silanol groups on the silica surface may be another factor causing variability of retention. The theoretical pKa value of silanol groups is 6.8 ± 0.5 (32) or 7.1 ± 0.5, but practically it varies in the range of 1.5 to 10.[33,34] This means that in applied conditions most, but probably not all, silanol groups may be suppressed. This possibility should be regarded, however, as very remote, since in applied conditions (acidic mobile phase containing amine modifier) most of the silanol effects were eliminated.[12,19,21]

CONCLUSIONS

1. RI data obtained in two labs in standardized conditions showed generally satisfactory reproducibility, allowing interlaboratory use and exchange of data. A search window of ±30 RI units is recommended for the use of data obtained with a different column in identical conditions. Within one laboratory or exactly the same system the search window of 10 RI units is justified.

2. The use of different HPLC conditions, especially a different pH of the mobile phase, may influence the RI values of some compounds, particularly those with pKa values around the pH of the mobile phase. Therefore, the importance of an exact standardization of HPLC conditions for interlaboratory exchange of RI-data was confirmed. However, the majority of retention data from all three libraries showed good agreement. The RI data from RI-libraries obtained in similar, but not exactly identical conditions may be used with caution.

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