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INFLUENCE OF REFERENCE SUBSTANCES ON RETENTION BEHAVIOUR OF HOMOLOGOUS COMPOUNDS ON STATIONARY PHASES OF INCREASING POLAR CHARACTER

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SUMMARY

The present work considers the effect of increased stationary phase polarity on the retention of homologous esters. The use of *n*-paraffins as a reference series with polar compounds is discussed together with the use of an ester series as complementary reference materials rather than other series that have been proposed for use with polar compounds.

INTRODUCTION

It has been observed qualitatively since the inception of gas chromatography that the retention ratio of two successive homologs, *i.e.* separation factor, is lower on a polar than on a non-polar stationary phase[1,2] and that in both cases the ratio decreases as the column temperature is increased[2,3]. While a decrease in retention ratio with increased stationary phase polarity has been explained[4] in terms of the thermodynamic properties of the sorption system, the availability of data of series of homologous esters on stationary phases which vary in character from essentially non-polar to those of considerable polar character with donor and acceptor properties allows a detailed study of the phenomena.

Retention increments of esters, *i.e.* formates to hexanoates, are reduced with an increase in the chain-length of the acidic reactant irrespective of the polarity of the phase but as the phase polarity is increased the position is not as clear[5]. The retention increments are dependent on the slopes of plots of the various series, as are retention ratios which are reduced as the slopes become shallower.

The present work considers the effect of increased stationary phase polarity on the retention of homologous esters. The use of *n*-paraffins as a reference series with polar compounds is discussed together with the use of an ester series as complementary reference materials rather than other series that have been proposed for use with polar compounds[6–9].

EXPERIMENTAL

The retention data used were obtained isothermally at 150 ° using 12 ft. $\times \frac{1}{4}$ in.

O.D. aluminium columns with 10% stationary phase on 62–72 mesh acid-washed and silanised Celatom as previously reported[5].

The slopes of the homologous series shown were calculated from the line of best fit determined by regression analysis using a simple programmable calculator. Kováts indices and equivalent indices using homologous esters as the reference series were similarly determined mathematically.

RESULTS AND DISCUSSION

The slopes of retention plots of homologous esters, *i.e.* formates to hexanoates on fourteen stationary phases, are shown in Table I. With all of the phases the decrease in slope indicated by the reduced retention increments[5] from formates to hexanoates is apparent independent of any consideration of the stationary phase.

TABLE I

SLOPES OF RETENTION PLOTS FOR *n*-ACID ESTER SERIES

Stationary phase	Ester homologs					
	Formate	Acetate	Propionate	Butyrate	Pentanoate	Hexanoate
SE-30	0.563	0.540	0.520	0.514	0.503	0.493
OV-7	0.588	0.566	0.551	0.534	0.521	0.517
DC-710	0.593	0.560	0.549	0.532	0.522	0.512
OV-25	0.532	0.509	0.504	0.491	0.486	0.478
100% Phenyl	0.493	0.488	0.487	0.477	0.473	0.472
DC-230	0.575	0.573	0.570	0.540	0.528	0.519
DC-530	0.506	0.509	0.498	0.490	0.480	0.476
XE-60	0.488	0.476	0.472	0.461	0.453	0.435
XF-1150	0.439	0.434	0.420	0.409	0.400	0.395
OV-225	0.502	0.481	0.488	0.467	0.452	0.444
SILAR 5CP	0.472	0.460	0.448	0.438	0.431	0.422
F-400	0.521	0.522	0.509	0.495	0.489	0.484
F-500	0.509	0.511	0.504	0.494	0.483	0.478
QF-1	0.449	0.452	0.440	0.426	0.418	0.410

With the siloxane phases that might be classed as donor materials, *i.e.* phenyl-substituted OV-7, DC-710, 100% phenyl, and aminoalkyl-substituted DC-530, there is a decrease in slope with increasing polarity. These data are shown in Fig. 1, while plots of the esters on acceptor phases are shown in Fig. 2. The plots vary slightly as the stationary phase polarity is increased; the separation, *i.e.* difference in intercepts, of the acid homologs is decreased, especially with the simpler esters on the more polar phases and similarly the slopes of the homologs tend to decrease with increased polarity as well as with increased length of the acid chain. Similar effects with acceptor phases have been described in terms of acceptor interactions[10]. The slopes of the ester homologs on an aminoalkyl-substituted polysiloxane, *i.e.* DC-530, and the separation of the lower homologs decreased in relation to a phenyl-substituted phase of equivalent polarity, *i.e.* DC-710, and the behaviour was similar to a slightly more polar acceptor phase, F-500. From the acceptor point of view the slopes and intercepts with DC-530 would have been expected to have increased or remained constant. Results
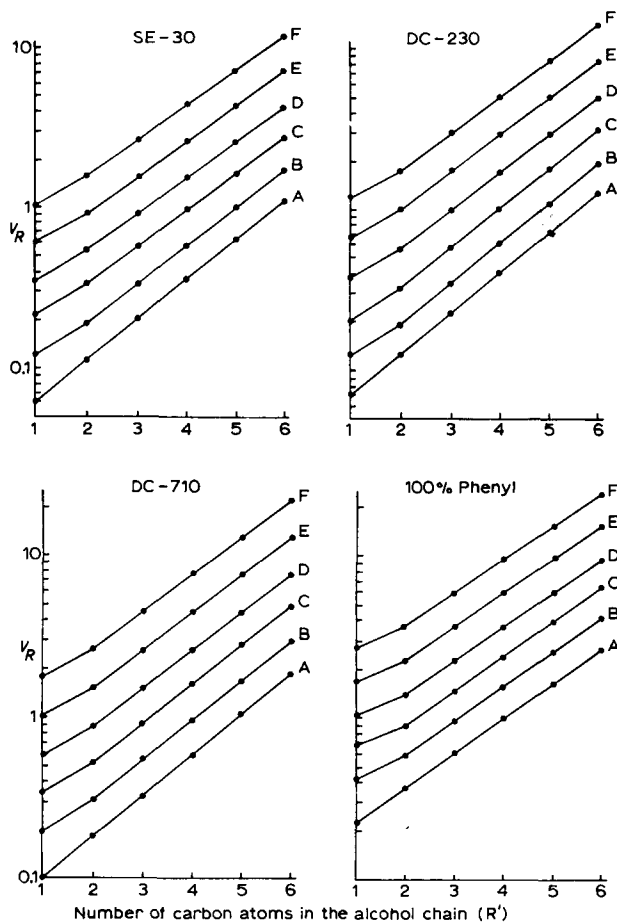
Fig. 1. Plot of logarithm of relative retention *versus* the number of carbon atoms in the alcohol chain of *n*-alkyl esters, where A = formate, B = acetate, C = propionate, D = butyrate, and E = pentanoate, on non-polar and donor stationary phases.

on an aminoalkyl phase of higher per cent substitution than DC-530 would have been desirable for examination, but it is considered that the behaviour of such a material would have been essentially similar to the higher polarity acceptor phases. A probable reason for the change in behaviour of stationary phases containing more polar donor and acceptor groups is that they tend to be more highly polarizable, especially with esters, than stationary phases containing donor phenyl groups, which in these environments tend to behave essentially as non-polar hydrocarbons due to weaker π bond interactions. For lower members of the ester series, the weak donating effect of the methyl group is capable of interacting with the acceptor carboxyl group, making a more polarizable molecule and one where less shielding of the carboxyl group can occur. With polar phases containing highly polarizable aminoalkyl or cyanoethyl groups these lower members would be more highly polarized and exhibit an increased retention with respect to the higher members with these phases. As the methyl group
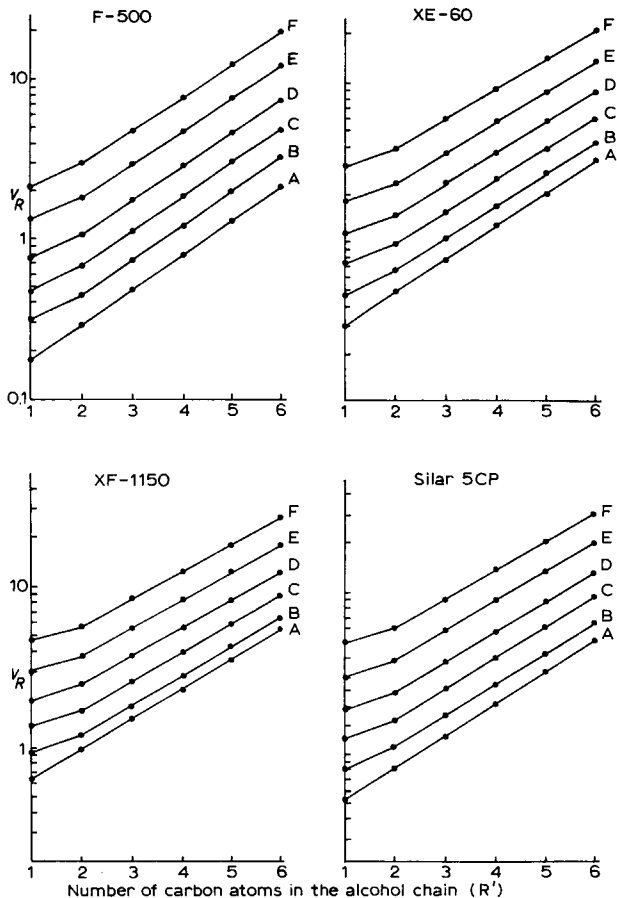
Fig. 2. Plot of logarithm of relative retention *versus* the number of carbon atoms in the alcohol chain of *n*-alkyl esters, where A = formate, B = acetate, C = propionate, D = butyrate, and E = pentanoate, on acceptor stationary phases.

or donor hydrogen moves further away from the carboxyl group due to the introduction of methylene groups in the chain, this increased retention would not be observed to the same extent, as the polarizability of the ester molecule would decrease[11,12], and the steric hindrance increase to a point where further methylene groups would have very little effect on the polarizability of the system.

The increase in slope or retention increment with increased polarity as shown in Table I is much clearer than in our earlier work[5]. The apparent anomaly is due to the fact that the values in Table I are the slopes of the line of best for the relative retention data relative to *n*-nonane, while the values shown previously are the slopes of the lines of best fit for the Kováts indices and hence are the relative retention data in comparison to *n*-hydrocarbons. Thus, if the slope of the hydrocarbon line differs from the slopes of the ester series the Kováts index increments will be either greater or smaller than 100 and will only be 100 if the slope of the hydrocarbon plot is parellel to the ester series considered. The slopes of the hydrocarbon plots vary with

TABLE II

CORRELATION OF POLARITY WITH HYDROCARBON AT 150°

Stationary phase	General polarity	Increasing order of polarity	Hydrocarbon slope
SE-30	0	1	0.545
OV-7	408	3	0.555
DC-710	674	6	0.557
OV-25	991	8	0.542
100% Phenyl	1313	9	0.524
DC-230	158	2	0.552
DC-530	631	5	0.514
XE-60	1619	11	0.470
XF-1150	2484	14	0.473
OV-225	1699	12	0.487
SILAR 5CP	2283	13	0.479
F-400	598	4	0.533
F-500	831	7	0.509
QF-1	1452	10	0.438

different phases and in Table II a measure of polarity of the phases is correlated with hydrocarbon plots where polarity is derived from McReynolds/Rohrschneider constants. The Kováts indices for the standard substances are summed for each phase, and from this sum is subtracted the sum for SE-30. Thus the magnitude of this value is a measure of general polarity of the phase and in this case is probably a more meaningful measure of polarity than a consideration of individual polarity factors. The general polarity will be dependent on the nature of the standard substances chosen; however, if a sufficient range of substances are included in the summation the order of polarity of the phases would be reasonably consistent, regardless of the nature of the substances. Table II would indicate that the slope of the hydrocarbon plot is not necessarily a measure of stationary phase polarity, especially with the donor phenyl-substituted phases, although in general the more polar the phase, the shallower the slope of the hydrocarbon plot. This means that as the values in Table I decrease with polarity the slopes of the hydrocarbon plots similarly decrease and trends with the Kováts indices increments will be less apparent, unless the slope of the esters plot decreases considerably as with XF-1150.

Associated with the use of Kováts indices is the reduced retention of hydrocarbons on highly polar phases and the resulting comparison of low-boiling polar solutes with n-alkanes of much higher boiling points. This problem has been considered by several workers and various alternate reference materials have been proposed. Grobler[6] has suggested the use of primary straight-chain alcohols as a second retention index system but the suggestion is without merit. Hawkes[7] has indicated that the n-alkanols provide the opposite extreme to the n-alkanes such that on polar phases many compounds would have much lower boiling points than the alcohols with which they are chromatographed and the n-propyl ethers were recommended as reference materials with ketones and aldehydes as other alternatives.

A carbon number system using the 2-alkanones has been reported by Dymond and Kilburn[8] and the merits of using these ketones as secondary references standards have been detailed by Ackman[9].

TABLE III

KOVÁTS INDICES AND INDICES RELATIVE TO ACETATE AND ESTERS (R = R') ON POLYSILOXANE

I = Kováts indices (relative to n-paraffins); I_A = indices relative to acetate esters; I_S = indices relative to symmetric esters (R = R').

	SE-30			OV-7			DC-710			OV-25			100% Phenyl			DC-230		
	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S
Benzene	674	476	479	719	465	471	767	469	476	830	466	470	860	454	462	688	478	484
Butanol	644	446	447	706	452	457	760	462	468	812	446	450	869	469	477	683	453	457
2-Pentanone	674	476	479	746	491	499	793	492	504	843	481	486	917	517	527	701	485	491
Nitropropane	718	523	528	832	576	588	892	597	611	961	611	620	1018	630	644	771	555	564
Pyridine	750	555	562	865	609	623	922	627	642	1005	661	671	1117	725	743	809	598	609
2-Methyl-2-pentanol	722	527	532	777	522	531	800	503	512	849	490	490	881	480	489	685	475	480
tert.-Butyl acetate	686	489	493	731	476	483	761	463	469	795	429	429	833	423	430	743	535	542
Methyl formate	386	180	168	453	199	193	496	193	185	571	178	173	618	184	182	419	211	203
Ethyl formate	495	292	286	552	298	296	585	285	282	715	272	270	715	292	293	521	312	309
Propyl formate	602	402	401	667	412	416	696	397	400	756	384	386	813	406	407	626	417	419
Butyl formate	707	511	516	774	518	527	804	507	516	852	490	496	907	506	516	734	525	532
Pentyl formate	810	617	627	879	624	637	912	617	631	952	602	611	998	608	622	835	625	638
Hexyl formate	907	718	733	976	720	738	1011	718	738	1047	708	720	1092	713	731	937	726	744
Methyl acetate	509	307	301	559	305	304	609	308	306	693	314	313	732	311	314	519	311	308
Ethyl acetate	592	392	391	648	393	396	690	391	393	760	388	390	803	390	396	599	390	391
Propyl acetate	695	498	502	755	500	508	796	498	507	853	492	497	895	493	503	704	495	501
Butyl acetate	794	601	610	855	600	621	895	600	607	991	600	614	991	600	614	810	600	611
Pentyl acetate	891	701	715	958	703	720	996	703	722	1044	704	716	1081	701	718	914	703	720
Hexyl acetate	988	801	820	1056	800	822	1092	800	825	1133	803	813	1174	804	825	1012	802	823
Methyl propionate	617	418	417	667	412	416	710	411	415	775	405	407	829	420	436	610	401	402
Ethyl propionate	692	496	500	743	388	496	783	486	493	845	483	487	885	481	490	692	482	488
Propyl propionate	789	599	608	851	595	608	884	589	602	934	587	595	977	585	597	792	584	595
Butyl propionate	886	696	710	949	694	711	984	691	709	1032	691	703	1069	687	703	896	686	701
Pentyl propionate	980	793	812	1047	791	813	1082	791	815	1125	795	810	1166	795	816	1003	793	813
Hexyl propionate	1074	891	913	1142	885	912	1176	886	916	1215	895	913	1255	894	919	1100	889	915
Methyl butyrate	702	505	510	767	512	520	803	506	515	868	508	514	907	506	516	719	510	517
Ethyl butyrate	778	584	592	840	585	597	876	581	593	931	579	587	968	575	587	794	585	595
Propyl butyrate	875	684	697	938	682	699	973	679	697	1022	680	692	1056	672	688	896	686	701
Butyl butyrate	969	782	800	1036	780	799	1069	777	801	1114	782	797	1147	774	794	994	783	804
Pentyl butyrate	1062	878	901	1131	875	900	1164	874	903	1205	883	901	1241	879	903	1091	879	905
Hexyl butyrate	1156	975	1002	1225	968	998	1258	969	1003	1292	981	1002	1331	979	1007	1186	974	1004
Methyl pentanoate	807	614	623	871	616	630	907	612	626	961	611	620	1001	611	625	821	611	623
Ethyl pentanoate	876	686	699	941	685	702	975	681	699	1024	682	694	1060	676	693	896	686	701
Propyl pentanoate	971	784	802	1036	779	800	1069	777	800	1114	782	797	1147	774	794	994	783	803
Butyl pentanoate	1063	879	902	1130	873	899	1163	873	901	1205	883	901	1237	875	899	1089	878	903
Pentyl pentanoate	1155	972	999	1222	965	996	1257	968	1002	1294	982	1004	1329	977	1004	1185	973	1003
Hexyl pentanoate	1247	1069	1101	1316	1059	1094	1349	1062	1101	1382	1080	1105	1420	1078	1100	1287	1066	1101
Methyl hexanoate	902	712	727	974	718	736	1006	713	733	1056	718	730	1091	712	729	925	715	732
Ethyl hexanoate	976	789	807	1045	789	811	1073	781	805	1118	787	802	1148	747	795	995	784	805
Propyl hexanoate	1064	880	903	1138	881	907	1164	874	903	1207	885	903	1239	876	900	1090	879	904
Butyl hexanoate	1156	974	1002	1231	974	1005	1256	968	1002	1295	983	1005	1329	977	1004	1184	972	1002
Pentyl hexanoate	1246	1068	1100	1325	1067	1102	1349	1063	1102	1384	1082	1107	1419	1078	1109	1278	1065	1100
Hexyl hexanoate	1347	1162	1199	1417	1159	1199	1440	1156	1200	1470	1178	1206	1508	1177	1212	1371	1158	1197

The methyl esters of the linear saturated acids form the basis of the carbon number system (CN) or the independently developed equivalent chain-length system (ECL) developed by Woodford and Van [Gent[13] and by Miwa and his co-workers[14], respectively. The esters are equivalent to the hydrocarbons used by Kováts and have found acceptance with lipid chemists although a refinement of the scheme em-

STATIONARY PHASES

DC-530			XE-60			XF-1150			OV-225			SILAR 5CP			F-400			F-500			QF-1		
I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S	I	I_A	I_S
733	483	486	863	439	450	1014	559	469	898	457	472	998	473	485	720	411	415	761	407	413	802	339	354
829	483	486	969	544	562	1146	612	633	977	541	559	1090	572	591	715	406	409	763	409	415	861	398	408
854	609	617	971	555	573	1139	606	627	986	549	569	1089	571	590	801	497	505	864	514	525	1017	553	593
829	583	590	1159	739	769	1379	854	894	1150	720	747	1291	790	821	887	588	600	960	614	629	1151	685	715
846	600	608	1113	692	720	1289	777	812	1148	714	741	1275	773	804	880	580	592	943	596	610	1081	616	640
842	596	604	984	561	580	1115	575	594	978	541	559	1073	554	571	794	494	502	838	487	496	918	454	468
729	478	482	857	432	443	972	410	417	873	431	444	947	417	426	776	469	475	815	464	472	909	445	458
488	227	221	641	213	210	817	232	224	682	232	235	762	217	214	509	204	198	563	201	198	689	227	225
587	329	327	754	328	332	907	335	335	755	308	315	860	324	327	604	301	299	658	300	301	780	317	322
686	434	435	848	424	434	1003	446	454	862	420	433	955	426	436	705	403	407	751	405	410	883	420	431
792	544	549	960	538	555	1092	549	566	967	530	548	1059	538	554	810	510	519	860	509	520	984	520	538
885	641	650	1064	642	667	1187	658	684	1070	637	661	1156	644	667	912	614	627	954	607	622	1089	624	649
980	740	753	1166	746	777	1279	765	799	1167	740	768	1252	747	776	1013	716	735	1059	717	737	1189	724	749
569	311	308	737	310	313	894	321	320	759	312	319	850	312	315	617	314	313	672	314	316	772	309	313
642	388	388	815	390	398	927	382	386	835	391	403	923	391	399	687	385	387	742	387	392	855	392	402
747	497	501	920	496	511	1040	489	501	932	494	510	1013	489	502	796	496	504	845	494	504	960	496	512
846	601	609	1022	600	621	1135	599	620	1030	596	617	1116	601	621	899	601	613	945	599	613	1064	599	623
942	701	712	1122	704	729	1227	705	734	1132	703	730	1212	704	730	999	702	720	1045	702	721	1169	703	734
1040	803	818	1221	803	837	1313	805	841	1229	804	836	1303	803	835	1098	803	825	1143	804	827	1267	801	838
663	409	410	837	413	422	976	415	421	851	409	421	947	418	426	904	412	416	754	400	406	842	387	397
740	489	493	897	473	487	1023	469	469	905	465	479	998	472	485	781	481	488	827	475	484	930	466	481
835	589	596	1003	581	601	1115	576	595	1013	578	599	1098	580	599	883	585	597	928	580	594	1033	568	590
934	693	704	1104	683	710	1204	679	705	1113	683	709	1195	685	710	984	687	704	1028	684	702	1133	667	695
1031	793	808	1202	782	816	1292	781	815	1211	785	816	1289	787	818	1083	788	810	1127	787	809	1234	768	803
1126	893	912	1300	883	922	1379	881	924	1307	886	922	1378	884	921	1181	887	914	1222	886	913	1332	866	907
757	507	512	923	500	515	1053	504	518	938	500	516	1032	509	524	802	502	510	846	495	505	960	496	512
828	582	589	986	564	583	1107	566	585	999	563	583	1085	567	585	876	577	589	918	570	583	1037	573	594
924	682	693	1089	669	694	1192	665	691	1098	667	692	1179	668	693	971	674	690	1017	673	690	1136	671	699
1021	783	798	1187	767	799	1278	764	797	1193	766	796	1272	768	799	1070	775	796	1114	774	796	1234	768	803
1116	883	901	1284	866	904	1364	864	905	1288	866	901	1362	866	903	1168	874	900	1212	875	901	1331	865	906
1209	980	1002	1380	964	1009	1449	962	1011	1383	966	1006	1451	962	1004	1264	972	1003	1306	973	1003	1427	960	1007
863	618	626	1027	605	627	1145	611	632	1042	608	630	1131	616	637	906	608	621	960	614	628	1067	604	625
930	688	699	1085	664	690	1190	663	688	1105	674	700	1180	669	693	978	681	697	1021	677	695	1141	676	704
1023	785	800	1180	759	791	1277	762	797	1196	770	800	1271	768	798	1074	779	800	1116	776	798	1240	774	808
1116	882	901	1279	861	899	1360	859	900	1289	868	903	1361	865	902	1171	877	903	1212	876	902	1334	869	908
1210	980	1002	1375	959	1003	1445	958	1006	1382	965	1004	1451	963	1005	1267	975	1006	1307	975	1005	1429	962	1010
1304	1078	1104	1470	1055	1105	1530	1055	1111	1476	1064	1109	1539	1058	1106	1362	1072	1107	1400	1071	1106	1524	1056	1109
960	719	731	1133	710	738	1237	716	746	1140	711	738	1225	718	746	1003	707	724	1050	707	726	1162	697	727
1026	788	803	1196	776	809	1278	764	797	1203	777	808	1275	772	802	1075	779	801	1119	779	801	1231	765	800
1119	885	903	1283	866	904	1362	861	902	1295	870	905	1363	867	904	1173	876	902	1213	876	902	1326	859	900
1209	980	1002	1377	961	1005	1445	957	1006	1383	966	1006	1451	963	1005	1265	973	1003	1307	974	1004	1418	951	998
1304	1079	1105	1471	1056	1107	1529	1054	1110	1480	1063	1107	1539	1058	1106	1361	1070	1106	1402	1073	1107	1514	1046	1099
1397	1175	1205	1565	1152	1208	1613	1151	1214	1575	1159	1208	1626	1152	1206	1465	1166	1206	1494	1169	1208	1605	1137	1196

ploying the methyl esters of monounsaturated esters and providing MECL (modified equivalent chain-length) values has found little use[15].

The success of the ECL scheme with fatty esters, aldehydes and alcohols on both polar and non-polar stationary phases has prompted use of simple esters as secondary reference standards. The use of a calibration line using homologous esters

requires some arbitrary selection of the reference series. With the lower esters the retention of compounds may be such that the index value may be quite high while with higher ester series many retention values may fall below the intercept. Table III shows a comparison of Kováts indices of six series of homologous esters together with ester index values calculated using the acetates as a reference series and also a line of best fit drawn through retention values of esters where both alkyl chains are of the same length. While the slopes and intercepts of the plots are shown in Table IV, the plot of esters where $R = R'$ has been previously used to determine the carboxyl contribution of esters on various phases[10,16] and in the present work is of interest as it covers a wider range of retention values than any of the simpler series.

TABLE IV

SLOPE AND INTERCEPTS OF ACETATE AND SYMMETRIC ESTER PLOTS

Stationary phase	Acetate esters		Symmetric esters	
	Slope	Intercept	Slope	Intercept
SE-30	0.527	0.0240	0.502	0.0267
OV-7	0.557	0.0270	0.532	0.0294
DC-710	0.547	0.0362	0.519	0.0398
OV-25	0.488	0.0695	0.472	0.0732
100% Phenyl	0.470	0.0947	0.452	0.0999
DC-230	0.555	0.0218	0.528	0.0240
DC-530	0.492	0.0394	0.474	0.0420
XE-60	0.462	0.1099	0.435	0.1184
XF-1150	0.409	0.2546	0.379	0.2807
OV-225	0.464	0.1158	0.442	0.1200
SILAR 5CP	0.443	0.2004	0.418	0.2148
F-400	0.500	0.0490	0.477	0.5299
F-500	0.490	0.0669	0.470	0.0706
QF-1	0.440	0.1446	0.413	0.1544

The difficulty in selecting an acceptable reference series is readily apparent by an examination of Table III. If the Kováts indices for the simple n-alkyl acetates are considered on SE-30, it is apparent that methyl acetate (b.p. 57.1°)[17] has a Kováts index of 599, i.e. equivalent to n-pentane (b.p. 36.2°) while hexyl acetate (b.p. 169.2°) has an I value of 988, i.e. almost equivalent to n-decane (b.p. 174.0°). With this non-polar stationary phase the solutes are of higher boiling point than the comparable paraffins; however, with DC-710, a material of modest polarity, the situation is reversed with methyl acetate ($I = 609$) equivalent to n-hexane (b.p. 69.0°) and hexyl acetate ($I = 1092$) almost equivalent to undecane (b.p. 196°). With the weaker acceptor phases, i.e. XE-60 and OV-225, methyl acetate ($I = 737$) is equivalent to a boiling point of approximately 107° and hexyl acetate ($I = 1221$) is equivalent to a boiling point of approximately 220°, while with the more polar acceptor phases methyl and hexyl acetates are equivalent to n-paraffins of boiling points approximating 140° and 235°. The much greater variations in boiling points at either end of the scale reflect the differences in the slopes of the paraffins and the esters on the various phases.

By considering the esters on a calibration line where methyl acetate with a carbon number of 3 has a nominal value of 300 and the higher acetates increase by 100 units per carbon number, a more realistic situation is achieved. The retention behaviour of a particular ester is immediately evident by simple comparison in the same way as is possible with ECL values. A line of best fit is constructed mathematically through the acetate data and accordingly some of the points, particularly those of the methyl and ethyl esters, show some deviations due to the non-linearity of the plots in this region. Retention data for the esters are also shown in Table III relative to a line of best fit of esters with the same number of carbon atoms on each side of the group, *i.e.* R = R′ = 1 = methyl acetate with an assigned value = 300. The index values are slightly higher than with an acetate calibration; however, from Table IV it is evident that while the slopes of the plots of the symmetric esters are somewhat lower than for the acetate series, the intercepts are greater. Both polar(ester) calibration lines are more suitable for use with polar solutes than the normal paraffins, proposals that have been suggested previously by Lorenz and Rogers[18] and by VandenHeuvel and Horning[19]. While the ester materials as secondary reference are more suitable, as suggested, than highly polar materials, it is unlikely that any single series could be satisfactory for all solutes. There is no reason, however, that retention relative to several reference series should not become more popular and be produced without difficulty with the increasing use of mini computers as data processing units.

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