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EMPIRICAL QUANTUM CHEMICAL APPROACH TO STRUCTURE–GAS CHROMATOGRAPHIC RETENTION INDEX RELATIONSHIPS

I. STEROL ACETATES

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SUMMARY

It has been found possible to correlate chromatographic retention parameters directly with molecular electronic structure by means of a linear relationship. The empirical parameters used are total energy and localized charge, calculated by the method of Del Re. They are deduced directly from the molecular structure and they permit the retention index to be calculated theoretically with an acceptable error.

INTRODUCTION

Many attempts have been made to establish a relationship between molecular structure and chromatographic retention parameters. Some of the results appear to be very hopeful, especially the system of characterization of stationary phases developed by McReynolds[1] and Rohrschneider[2], the techniques based on factor analysis[3], and particularly the work of Takacs and co-workers[4–8] for the assignment of structural parameters of the solutes.

In general, the above methods involve the expression of retention as the sum of structural contributions, or as a function of different physical properties, by means of a multilinear correlation technique (boiling point, ΔH_v, parachor, dipole moment, refractive index, etc.[9–11]). All the methods described in the literature are a useful contribution to the *a priori* determination of chromatographic retention parameters. However, in spite of the utility of the results obtained, a general method for possible application to different stationary phases and to compounds of different structures is not yet available.

The optimum solution would be the development of a method that fulfils the following requirements: (a) applicability to any type of molecular structure; (b) capable of being used as a basis for a physical explanation of retention phenomena;

(c) satisfactory accuracy; and (d) starting data determined by direct evaluation. Therefore, the parameters used in the determination of the retention index must be calculated independent of the experimental chromatographic determination, *i.e.*, from the actual molecular structure.

EXPERIMENTAL

In the proposed method, the starting point is that a molecule will be eluted more or less quickly according to (a) its volatility, which can be related to its molecular volume or size, and (b) the interaction forces upon the molecule during the elution process, *i.e.*, interactions with the stationary phase or support (Van der Waals, Keeson, Debye, London, electrostatic, hydrogen bond, dipole moment, etc.), which, in a first-order approach, will depend on the distribution of electronic charge in the molecule. For this reason, in the present work the possibility of applying one of the methods developed in quantum chemistry, allowing the calculation of this charge distribution, as well as some parameters that are directly related to the steric effect of the substituents, is considered.

The method used in this work was proposed by Del Re[12–14] and Carbó[15] and is an extension to non-conjugate systems of the LCAO–MO method in the Hückel[16] approach; it has been widely applied in other areas such as pharmacology and drug design[17–22]. In this system, for a given stationary phase at a fixed temperature the retention index can be expressed as

$$RI = E \cdot a + \Sigma Q_i \cdot c_i + b \qquad (1)$$

where a, b and c_i are coefficients that can be calculated by multilinear correlation, E is a dimensionless parameter that is a function of the molecular volume, and the total electronic energy (E) selected in this work is obtained by Del Re's method, and Q_i is the effective charge located on the ith atom of the molecule.

The summation in eqn. 1 refers to the set of charges $\{Q_i\}$ that presents the most appreciable *a priori* variations, due to some structural change in the molecular set.

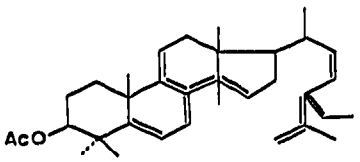
RESULTS AND DISCUSSION

The charge distributions in 59 compounds derived from cholestanol acetate were calculated by means of an IBM 1130 computer. Bibliographic retention data were taken for the family of sterol acetates from a paper by Patterson[23], who gave retention times relative to cholesterol acetate for 92 sterol acetates in four different stationary phases. The retention indices were deduced for each molecule and in each of the stationary phases, removing the isomers with identical properties.

Fig. 1 summarizes all the functional groups for the 59 structures studied. The carbon atoms whose effective charge will give greater differences in molecular structure for the whole group considered may be easily observed.

In accordance with the above considerations, we have selected the carbon atoms indicated by spots in Fig. 2 (positions 4, 5, 6, 7, 8, 9, 14, 15, 22, 24, 25 and 26), as being highly significant centres related to the variations in the retention index.

In Fig. 3 are given the Q_i and E values, obtained for each sterol acetate, that



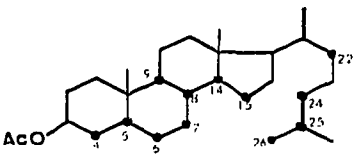


Fig. 1. Summary of all functional groups for the 59 structures studied.

Fig. 2. The carbon atoms which are the significant centres in relation with variations in the retention index.

Compound (*E4 = × 10⁴)	E	Q4 *E4	Q5 *E4	Q6 *E4	Q7 *E4	Q8 *E4	Q9 *E4	Q14 *E4	Q15 *E4	Q22 *E4	Q24 *E4	Q25 *E4	Q26 *E4
5,22-Cholestadienol	177.25	−711	174	−234	−818	−357	−358	−354	−761	−247	−813	−311	−1185
5,22,24-Cholestatrienol	172.76	−711	174	−234	−818	−357	−358	−354	−761	−241	−298	192	−1233
5,7,22-Cholestatrienol	172.76	−713	165	−301	−303	143	−410	−404	−768	−247	−813	−311	−1185
5-Cholestenol	181.77	−711	174	−234	−818	−357	−358	−354	−761	−758	−755	−303	−1184
Cholestenol	186.27	−661	−329	−759	−760	−348	−352	−353	−761	−758	−755	−303	−1184
8,14-Cholestadienol	177.29	−662	−335	−767	−815	100	149	88	−243	−758	−755	−303	−1184
14-Methyl-8-cholestenol	188.39	−662	−335	−767	−810	154	155	25	−772	−758	−755	−303	−1184
8-Cholestenol	181.78	−662	−335	−767	−809	160	156	−403	−768	−758	−755	−303	−1184
5,25-Cholestadienol	177.24	−711	174	−234	−818	−357	−358	−354	−761	−759	−807	189	−637
5,24-Cholestadienol	177.26	−711	174	−234	−818	−357	−358	−354	−761	−765	−229	200	−1233
5,7-Cholestadienol	177.28	−713	165	−301	−303	143	−410	−404	−768	−758	−755	−303	−1184
24-Methyl-5,22-cholestadienol	183.86	−711	−174	−234	−818	−357	−358	−354	−761	−241	−379	−317	−1186
7-Cholestenol	181.77	−662	−337	−816	−235	151	−404	−404	−768	−758	−755	−303	−1184
24-Methyl-7,14,22-cholestatrienol	179.37	−662	−337	−817	−242	91	−409	87	−243	−241	−379	−317	−1186
8,24-Cholestadienol	177.27	−662	−335	−767	−809	160	156	−403	−768	−765	−229	200	−1233
14,24-Dimethyl-8,22-cholestadienol	190.49	−662	−335	−767	−810	154	155	25	−772	−241	−379	−317	−1186
14-Methyl-7-cholestenol	188.38	−662	−337	−816	−237	146	−404	24	−772	−758	−755	−303	−1184
24-Methyl-5-cholestenol	188.37	−711	174	−324	−818	−357	−358	−354	−761	−759	−321	−310	−1185
14,24-Dimethyl-8,24(28)-cholestadienol	190.48	−662	−335	−767	−810	154	155	25	−772	−766	170	−364	−1191
24-Methyl-8,14-cholestadienol	183.90	−662	−335	−767	−815	100	149	88	−243	−759	−321	−310	−1185
24-Methyl cholestanol	192.87	−661	−329	−759	−760	−348	−352	−353	−761	−759	−321	−310	−1186
14,24-Dimethyl-8-cholestenol	195.00	−662	−335	−767	−810	154	155	25	−772	−759	−321	−310	−1185
24-Methyl-8-cholestenol	188.39	−662	−335	−767	−809	60	156	−403	−768	−759	−321	−310	−1185
4,4,14-Trimethyl-8-cholestenol	201.60	207	−351	−769	−810	154	155	25	−772	−758	−755	−303	−1184
24-Methyl-5,7-cholestadienol	183.88	−713	165	−301	−303	143	−410	−404	−768	−759	−321	−310	−1185
24-Ethyl-5,22-cholestadienol	190.46	−711	174	−234	−818	−357	−358	−354	−761	−241	−390	−318	−1186
24-Methyl-7,24(28)-cholestadienol	183.86	−662	−337	−816	−235	151	−404	−404	−768	−766	170	−364	−1191
24-Methyl-7-cholestenol	188.38	−662	−337	−816	−235	151	−404	−404	−768	−759	−321	−310	−1185
4,14,24-Trimethyl-8,24(28)-cholestadienol	197.08	−225	−343	−768	−810	154	155	25	−772	−766	170	−364	−1191
24-Methyl-7,9(11),22-cholestatrienol	179.37	−664	−343	−817	−242	91	88	−410	−769	−241	−379	−317	−1186
24-Methyl-5,7,22-cholestatrienol	179.37	−713	165	−301	−303	143	−410	−404	−768	−241	−379	−317	−1186
24-Methyl-7,22-cholestadienol	183.86	−662	−337	−816	−235	−151	−404	−404	−768	−241	−379	−317	−1186
24-Methyl-5,24(28)-cholestadienol	183.86	−711	174	−234	−818	−357	−358	−354	−761	−766	170	−364	−1191
24-Methyl-8(14)-cholestenol	188.39	−661	−330	−766	−809	160	−403	155	−810	−759	−321	−310	−1185
14,24-Dimethyl-7,22-cholestadienol	190.47	−662	−337	−816	−237	146	−404	24	−772	−241	−379	−317	−1186
4,14,24-Trimethyl-8-cholestenol	201.60	−225	−343	−768	−810	154	155	25	−772	−759	−321	−310	−1185
4,24-Dimethyl-8,14-cholestadienol	190.50	−225	−343	−768	−816	100	149	88	−243	−759	−321	−310	−1185
14,24-Dimethyl-7-cholestenol	194.99	−662	−337	−816	−237	146	−404	24	−772	−759	−321	−310	−1185
4,4,14-Trimethyl-8,24-cholestadienol	197.09	207	−351	−769	−810	154	155	25	−772	−765	−229	−200	−1233
24-Ethyl-5,7,22-cholestatrienol	185.98	−713	165	−301	−303	143	−410	−404	−768	−241	−390	−318	−1185
4,24-Dimethyl-8-cholestenol	194.99	−225	−343	−768	−810	160	155	−403	−768	−759	−321	−310	−1185
24-Ethyl-5,25-cholestadienol	190.46	−711	174	−234	−818	−358	−358	−354	−761	−759	−384	180	−638
24-Ethyl-7,22,25-cholestatrienol	185.95	−662	−337	−816	−235	151	−404	−404	−768	−242	−443	171	−640
24-Ethyl-7,22-cholestadienol	190.47	−662	−337	−816	−235	151	−404	−404	−768	−241	−390	−318	−1186
24-Methyl-8,24(28)-cholestadienol	183.87	−662	−335	−767	−809	−160	−156	−403	−768	−766	170	−364	−1191
24-Ethyl-8(14)-cholestenol	194.99	−661	−330	−766	−809	160	−403	155	−810	−759	−330	−312	−1185
24-Ethyl-5-cholestenol	194.98	−711	174	−234	−818	−357	−358	−354	−761	−759	−330	−312	−1185
24-Ethyl-5,24(28)-cholestadienol	185.97	−711	174	−234	−818	−357	−358	−354	−761	−248	101	−370	−1191
24-Ethyl-8,14-cholestadienol	190.50	−662	−335	−767	−815	100	−149	88	−243	−759	−330	−312	−1185
24-Ethyl cholestanol	199.48	−661	−329	−759	−760	−348	−352	−353	−761	−759	−330	−312	−1185
14-Methyl-24-ethyl-8-cholestenol	201.60	−662	−335	−767	−810	154	155	25	−772	−759	−330	−312	−1185
24-Ethyl-8-cholestenol	194.99	−662	−335	−767	−809	160	156	−403	−768	−759	−330	−312	−1185
24-Ethyl-8,14,24(28)-cholestatrienol	185.99	−662	−335	−767	−815	100	149	88	−243	−766	170	−364	−1191
24-Ethyl-7,25-cholestadienol	190.46	−662	−337	−816	−836	151	−404	−404	−768	−759	−384	180	−638
24-Ethyl-5,7-cholestadienol	190.49	−713	165	−301	303	143	−410	−404	−468	−759	−330	−312	−1185
24-Ethyl-7-cholestenol	194.98	−662	−337	−816	−235	151	−404	−404	−768	−759	−330	−312	−1185
4-Methyl-24-ethyl-8,14-cholestadienol	197.11	−225	−343	−768	−816	100	149	88	−243	−759	−330	−312	−1185
4,14-Dimethyl-24-ethyl-8-cholestenol	208.21	−225	−343	−768	−810	154	155	−25	−772	−759	−330	−312	−1185
4-Methyl-24-ethyl-8-cholestenol	201.60	−225	−343	−768	−810	160	155	−403	−768	−759	−330	−312	−1185

Fig. 3. Computer outprint giving Q_i and E values for each sterol acetate.

	Stationary phase	*SE-30*	*Stationary phase*	*QF-1*
	temperature	*244 C*	*temperature*	*231 C*
	Coefficients	*Signification*	*Coefficients*	*Signification*
1	6.905	100.00	27.335	100.00
2	−245.240	98.37	−10.788	0.00
3	352.222	82.61	2270.016	98.43
4	−31.770	22.36	−48.323	40.08
5	186.980	88.47	762.720	94.38
6	945.644	99.99	2762.298	99.99
7	−490.539	99.98	−807.274	96.42
8	1063.588	100.00	−2838.205	100.00
9	1430.304	100.00	3755.530	100.00
10	112.571	87.85	−384.662	86.45
11	420.608	99.99	2233.438	100.00
12	457.502	99.97	1078.707	99.11
13	144.658	74.55	1278.912	96.67
Independent term	2051.56		*Independent term*	−521.07

Compound	*RI*	*CI*	*ER*	*EM*	*RI*	*CI*	*ER*	*EM*
5,22-Cholestadienol	3160.5	3159.4	0.03	0.57	3706.6	3690.7	0.43	1.86
5,22,24-Cholestatrienol	3173.7	3172.4	0.04	0.64	3724.4	3730.7	0.16	0.73
5,7,22-Cholestatrienol	3183.0	3173.1	0.31	5.05	3813.3	3760.1	1.41	6.22
5-Cholestenol	3184.9	3187.6	0.08	1.36	3813.3	3847.7	0.89	4.03
Cholestenol	3190.6	3200.8	0.32	5.20	3857.7	3865.1	0.19	0.86
8,14-Cholestadienol	3190.6	3184.8	0.18	2.92	3786.6	3767.3	0.51	2.26
14-Methyl-8-cholestenol	3192.4	3197.1	0.14	2.35	3884.4	3904.3	0.51	2.33
8-Cholestenol	3194.3	3198.2	0.18	1.95	3822.2	3848.6	0.68	3.10
5,25-Cholestadienol	3198.1	3184.6	0.42	6.82	3920.0	3835.4*	2.20	9.90
5,24-Cholestadienol	3201.8	3200.8	0.03	0.52	3893.3	3890.0	0.08	0.39
5,7-Cholestadienol	3201.8	3201.3	0.01	0.25	3920.0	3917.1	0.07	0.33
24-Methyl-5,22-cholestadienol	3207.5	3210.8	0.10	1.67	3893.3	3888.1	0.13	0.60
7-Cholestenol	3207.5	3214.2	0.20	3.39	3911.1	3934.9	0.60	2.79
24-Methyl-7,14,22-cholestatrienol	3209.3	3236.2*	0.83	13.62	3875.5	3973.0*	2.45	11.42
8,24-Cholestadienol	3209.3	3211.3	0.06	1.01	3893.3	3890.9	0.06	0.28
14,24-Dimethyl-8,22-cholestadienol	3215.0	3232.5*	0.54	8.87	3964.4	4023.7*	1.47	6.95
14-Methyl-7-cholestenol	3216.9	3213.1	0.11	1.89	4000.0	3990.8	0.22	1.07
24-Methyl-5-cholestenol	3241.3	3251.4	0.31	5.16	4071.1	4124.8*	1.30	6.30
14,24-Dimethyl-8,24(28)-cholestadienol	3241.3	3247.4	0.18	3.12	4142.2	4160.9	0.44	2.18
24-Methyl-8,14-cholestadienol	3246.9	3248.3	0.04	0.71	4026.6	4044.0	0.43	2.03
24-Methyl cholestanol	3248.8	3264.3*	0.47	7.89	4124.4	4141.7	0.41	2.02
14,24-Dimethyl-8-cholestenol	3248.8	3260.6	0.36	6.00	4151.1	4181.0	0.71	3.51
24-Methyl-8-cholestenol	3252.5	3252.2	0.00	0.14	4171.1	4097.7*	1.79	8.59
4,4,14-Trimethyl-8-cholestenol	3261.9	3266.4	0.13	2.28	4257.7	4261.0	0.07	0.37
24-Methyl-5,7-cholestadienol	3263.8	3264.9	0.03	0.53	4213.3	4193.8	0.46	2.28
24-Ethyl-5,22-cholestadienol	3263.8	3268.1	0.13	2.18	4097.7	4145.1	1.14	5.55
24-Methyl-7,24(28)-cholestadienol	3263.8	3264.5	0.02	0.36	4168.8	4191.4	0.53	2.64
24-Methyl-7-cholestenol	3271.3	3277.7	0.19	3.23	4186.6	4211.6	0.59	2.92
4,14,24-Trimethyl-8,24(28)-cholestadienol	3273.2	3282.0*	0.26	4.46	4284.4	4338.9*	1.25	6.38
24-Methyl-7,9(11),22-cholestatrienol	3218.7	3189.3*	0.92	14.94	3911.1	3875.0	0.92	4.22
24-Methyl-5,7,22-cholestatrienol	3226.2	3236.7	0.32	5.30	4008.8	4036.5	0.68	3.24
24-Methyl-7,22-cholestadienol	3231.9	3221.0	0.33	5.51	3991.1	3970.8	0.50	2.37
24-Methyl-5,24(28)-cholestadienol	3233.8	3238.0	0.13	2.14	4062.2	4104.2*	1.02	4.92
24-Methyl-8(14)-cholestenol	3237.5	3223.8	0.42	6.96	4017.7	3997.1	0.51	2.42
14,24-Dimethyl-7,22-cholestadienol	3239.4	3248.5	0.28	4.62	4080.0	4110.1	0.73	3.53
4,14,24-Trimethyl-8-cholestenol	3278.8	3295.2*	0.49	8.29	4293.3	4359.1*	1.50	7.70
4,24-Dimethyl-8,14-cholestadienol	3278.8	3282.9	0.12	2.06	4151.1	4222.0*	1.67	8.31
14,24-Dimethyl-7-cholestenol	3282.6	3276.7	0.18	3.00	4284.4	4267.5	0.39	1.98
4,4,14-Trimethyl-8,24-cholestadienol	3286.3	3261.3*	0.76	12.69	4364.4	4260.1*	2.44	12.22
24-Ethyl-5,7,22-cholestatrienol	3286.3	3281.8	0.13	2.29	4231.1	4214.7	0.38	1.91
4,24-Dimethyl-8-cholestenol	3288.2	3296.3	0.24	4.11	4222.2	4303.4*	1.88	9.51
24-Ethyl-5,25-cholestadienol	3288.2	3293.1	0.14	2.48	4231.1	4289.7*	1.36	6.87
24-Ethyl-7,22,25-cholestatrienol	3292.0	3291.6	0.01	0.20	4195.5	4219.8	0.57	2.84
24-Ethyl-7,22-cholestadienol	3293.8	3294.6	0.02	0.40	4222.2	4232.3	0.23	1.18
24-Methyl-8,24(28)-cholestadienol	3246.9	3233.6	0.41	6.76	4071.1	4042.0	0.72	3.41
24-Ethyl-8(14)-cholestenol	3299.5	3268.9*	0.93	15.53	4257.7	4175.1*	1.97	9.68
24-Ethyl-5-cholestenol	3303.2	3296.2	0.21	3.54	4311.1	4302.4	0.20	1.00
24-Ethyl-5,24(28)-cholestadienol	3303.2	3255.2*	1.47	24.34	4257.7	4126.0*	3.19	15.43
24-Ethyl-8,14-cholestadienol	3308.9	3308.0	0.02	0.42	4240.0	4246.1	0.14	0.72
24-Ethyl cholestanol	3310.7	3309.4	0.03	0.65	4364.4	4319.9	1.03	5.21
14-Methyl-24-ethyl-8-cholestenol	3310.7	3305.7	0.15	2.55	4400.0	4359.1	0.93	4.79
24-Ethyl-8-cholestenol	3316.4	3306.8	0.29	4.86	4311.1	4303.4	0.17	0.90
24-Ethyl-8,14,24(28)-cholestatrienol	3318.3	3280.8*	1.14	18.98	4284.4	4204.7*	1.89	9.34
24-Ethyl-7,25-cholestadienol	3322.0	3331.0	0.26	4.53	4355.5	4331.4	0.55	2.82
24-Ethyl-5,7-cholestadienol	3331.4	3352.8*	0.63	10.87	4480.0	4484.5	0.10	0.53
24-Ethyl-7-cholestenol	3340.8	3322.8*	0.54	9.13	4444.4	4389.6*	1.24	6.41
4-Methyl-24-ethyl-8,14-cholestadienol	3348.3	3328.0*	0.61	10.30	4408.8	4400.0	0.19	1.03
4,14-Dimethyl-24-ethyl-8-cholestenol	3350.2	3345.6	0.13	2.33	4560.0	4551.3	0.18	1.01
4-Methyl-24-ethyl-8-cholestenol	3357.7	3341.4	0.48	8.25	4471.1	4481.4	0.23	1.21

Mean absolute error	5.68	Mean absolute error	19.22
Mean relative error	0.17	Mean relative error	0.47
Mean error to range	2.88	Mean error to range	2.25
Regression coefficient	0.9892	Regression coefficient	0.9945
t (student) of *R*	38.2136	*t* (student) of *R*	51.085
R signification	>99.99	*R* signification	>99.99
R square	0.9785	*R* square	0.9890
F(13, 32)	112.329	*F*(13, 29)	200.741
F signification	>99.99	*F* signification	>99.99
Exner's function	0.0943	Exner's function	0.0705

Stationary phase	*HIEFF8BP*
temperature	*238 C*
Coefficients	*Signification*
1 25.040	100.00
2 −1187.263	95.98
3 3070.667	94.76
4 −463.336	26.16
5 −555.624	57.39
6 7364.113	100.00
7 −4817.222	99.99
8 −7031.368	100.00
9 9981.538	100.00
10 −542.512	80.58
11 4562.798	100.00
12 2966.568	99.96
13 3906.810	99.99
Independent term 753.47	

Stationary phase	*PMPE*
temperature	*250 C*
Coefficients	*Signification*
1 23.383	100.00
2 −1752.951	98.94
3 2768.280	88.13
4 −2290.333	81.08
5 −775.351	67.48
6 6869.555	100.00
7 −4624.869	99.99
8 −8141.136	100.00
9 9381.873	100.00
10 −1.072	0.00
11 4505.205	100.00
12 3794.379	99.99
13 2822.691	99.63
Independent term 861.97	

Compound	*RI*	*CI*	*ER*	*EM*	*RI*	*CI*	*ER*	*EM*
5,22-Cholestadienol	3887.1	3871.4	0.40	1.30	3924.3	3926.7	0.06	0.19
5,22,24-Cholestatrienol	4369.2	4123.9*	5.94	20.43	4464.8	4230.8*	5.53	19.32
5,7,22-Cholestatrienol	4225.6	4154.0	1.72	5.96	4216.2	4198.2	0.42	1.48
5-Cholestenol	4010.2	4041.4	0.77	2.59	4064.8	4061.7	0.07	0.25
Cholestenol	4010.2	4017.9	0.19	0.64	4086.4	4137.4	1.23	4.20
8,14-Cholestadienol	4102.5	4091.3	0.27	0.93	4140.5	4135.9	0.11	0.37
14-Methyl-8-cholestenol	3948.7	3921.2	0.70	2.29	4000.0	3983.5	0.41	1.35
8-Cholestenol	4020.5	4065.4	1.10	3.74	4140.5	4185.7	1.07	3.73
5,25-Cholestadienol	4328.2	4263.7	1.51	5.36	4356.7	4273.3	1.95	6.88
5,24-Cholestadienol	4307.6	4298.5	0.21	0.76	4378.3	4369.8	0.19	0.70
5,7-Cholestadienol	4307.6	4324.0	0.37	1.36	4313.5	4333.3	0.45	1.63
24-Methyl-5,22-cholestadienol	4112.8	4125.1	0.29	1.02	4162.1	4177.4	0.36	1.26
7-Cholestenol	4225.6	4298.8	1.70	6.09	4345.9	4405.2	1.34	4.89
24-Methyl-7,14,22-cholestatrienol	4194.8	4515.0*	7.09	26.68	4259.4	4567.5*	6.74	25.44
8,24-Cholestadienol	4317.9	4322.5	0.10	0.38	4486.4	4493.8	0.16	0.60
14,24-Dimethyl-8,22-cholestadienol	4051.2	4111.7	1.47	5.04	4075.6	4195.5*	2.85	9.90
14-Methyl-7-cholestenol	4184.6	4154.7	0.71	2.48	4237.8	4203.3	0.82	2.84
24-Methyl-5-cholestenol	4338.4	4406.6	1.54	5.68	4378.3	4429.4	1.15	4.21
14,24-Dimethyl-8,24(28)-cholestadienol	4358.9	4375.1	0.37	1.35	4378.3	4424.0	1.03	3.77
24-Methyl-8,14-cholestadienol	4461.5	4452.3	0.20	0.76	4475.6	4483.0	0.16	0.60
24-Methyl cholestanol	4313.9	4378.6	1.47	5.38	4410.8	4484.2	1.63	6.06
14,24-Dimethyl-8-cholestenol	4246.1	4282.2	0.84	3.00	4281.0	4330.6	1.14	4.09
24-Methyl-8-cholestenol	4348.7	4352.8	0.09	0.34	4475.6	4464.1	0.25	0.95
4,4,14-Trimethyl-8-cholestenol	4143.5	4143.9	0.00	0.03	4151.3	4136.0	0.36	1.26
24-Methyl-5,7-cholestadienol	4717.9	4685.0	0.70	2.73	4713.5	4680.3	0.70	2.73
24-Ethyl-5,22-cholestadienol	4358.9	4392.2	0.75	2.77	4410.8	4422.9	0.27	0.99
24-Methyl-7,24(28)-cholestadienol	4769.2	4752.7	0.34	1.37	4886.4	4845.7	0.84	3.36
24-Methyl-7-cholestenol	4615.3	4659.8	0.95	3.70	4745.9	4752.3	0.13	0.52
4,14,24-Trimethyl-8,24(28)-cholestadienol	4482.0	4485.8	0.08	0.31	4400.0	4499.4*	2.21	8.21
24-Methyl-7,9(11),22-cholestatrienol	4256.4	4098.3*	3.85	13.16	4367.5	4247.3*	2.83	9.92
24-Methyl-5,7,22-cholestatrienol	4461.5	4514.6	1.17	4.45	4421.6	4245.3*	2.72	10.21
24-Methyl-7,22-cholestadienol	4338.4	4266.9	1.67	5.95	4464.8	4409.7	1.24	4.54
24-Methyl-5,24(28)-cholestadienol	4451.2	4495.3*	0.98	3.67	4486.4	4502.2	0.34	1.29
24-Methyl-8(14)-cholestenol	4276.9	4261.9	0.35	1.24	4356.7	4297.6	1.37	4.87
14,24-Dimethyl-7,22-cholestadienol	4276.9	4345.3	1.57	5.70	4335.1	4415.3	1.81	6.62
4,14,24-Trimethyl-8-cholestenol	4348.7	4392.9	1.00	3.68	4313.5	4406.0	2.10	7.64
4,24-Dimethyl-8,14-cholestadienol	4564.1	4563.1	0.02	0.08	4518.9	4558.5	0.86	3.27
14,24-Dimethyl-7-cholestenol	4543.5	4515.8	0.61	2.31	4594.5	4550.4	0.97	3.64
4,4,14-Trimethyl-8,24-cholestadienol	4492.3	4282.3*	4.90	17.49	4508.1	4292.4*	5.02	17.81
24-Ethyl-5,7,22-cholestatrienol	4758.9	4675.1	1.79	6.98	4724.3	4694.7	0.62	2.44
4,24-Dimethyl-8-cholestenol	4461.5	4537.7*	1.67	6.35	4508.1	4608.7*	2.18	8.31
24-Ethyl-5,25-cholestadienol	4707.6	4783.8	1.59	6.35	4702.7	4768.5	1.38	5.43
24-Ethyl-7,22,25-cholestatrienol	4871.7	4871.2	0.01	0.04	4983.7	4977.2	0.13	0.53
24-Ethyl-7,22-cholestadienol	4635.8	4649.5	0.29	1.14	4767.5	4766.4	0.02	0.09
24-Methyl-8,24(28)-cholestadienol	4482.0	4434.1	1.08	3.99	4572.9	4550.6	0.49	1.84
24-Ethyl-8(14)-cholestenol	4533.3	4422.1*	2.51	9.26	4627.0	4446.8*	4.05	14.88
24-Ethyl-5-cholestenol	4625.6	4562.6	1.38	5.24	4648.6	4557.9	1.98	7.48
24-Ethyl-5,24(28)-cholestadienol	4789.7	4486.9*	6.74	25.23	4800.0	4518.2*	6.23	23.27
24-Ethyl-8,14-cholestadienol	4769.2	4756.1	0.27	1.09	4767.5	4770.0	0.05	0.20
24-Ethyl cholestanol	4605.1	4539.1	1.45	5.49	4681.0	4633.6	1.02	3.91
14-Methyl-24-ethyl-8-cholestenol	4512.8	4442.4	1.58	5.86	4529.7	4479.8	1.11	4.12
24-Ethyl-8-cholestenol	4615.3	4586.7	0.62	2.38	4767.5	4681.9	1.82	7.07
24-Ethyl-8,14,24(28)-cholestatrienol	5025.6	4710.9*	6.68	26.22	5037.8	4731.1*	6.48	25.33
24-Ethyl-7,25-cholestadienol	5087.1	5075.3	0.23	0.98	5135.1	5159.2	0.46	1.98
24-Ethyl-5,7-cholestadienol	5087.1	5144.7	1.11	4.79	5016.2	5110.9	1.85	7.82
24-Ethyl-7-cholestenol	4964.1	4820.0*	2.98	12.00	5070.2	4901.4*	3.44	13.94
4-Methyl-24-ethyl-8,14-cholestadienol	4912.8	4723.2*	4.01	15.79	4810.8	4707.6	2.19	8.51
4,14-Dimethyl-24-ethyl-8-cholestenol	4615.3	4588.2	0.59	2.25	4562.1	4595.9	0.73	2.78
4-Methyl-24-ethyl-8-cholestenol	4717.9	4697.9	0.42	1.66	4789.1	4757.9	0.65	2.58

Mean absolute error	39.26	Mean absolute error	38.08
Mean relative error	0.89	Mean relative error	0.84
Mean error to range	3.27	Mean error to range	3.14
Regression coefficient	0.9879	Regression coefficient	0.9848
t (student) of *R*	37.2594	*t* (student) of *R*	32.6137
R signification	>99.99	*R* signification	>99.99
R square	0.9760	*R* square	0.9699
F(13, 34)	106.7899	*F*(13, 33)	81.8197
F signification	>99.99	*F* signification	>99.99
Exner's function	0.0965	Exner's function	0.110

Fig. 4. Computer outprint giving *RI* and *CI* values and relative errors with different stationary phases and column temperatures for each sterol acetate.

Stationary phase SE−30
temperature 244 C

	Coefficients	*Signification*
1	0.036	100.00
2	−1.306	98.37
3	1.876	82.62
4	−0.169	22.35
5	−0.995	88.47
6	5.035	99.99
7	−2.612	99.98
8	−5.663	100.00
9	7.615	100.00
10	0.599	87.86
11	2.239	99.99
12	2.436	99.97
13	0.769	74.52

Independent term
−5.03

Compound	*RT*	*CT*	*ER*	*EM*
5,22-Cholestadienol	0.870	0.864	0.69	0.57
5,22,24-Cholestatrienol	0.940	0.933	0.73	0.64
5,7,22-Cholestatrienol	0.990	0.936	5.66	5.05
5-Cholestenol	1.000	1.014	1.41	1.36
Cholestenol	1.030	1.084	5.03	5.20
8,14-Cholestadienol	1.030	0.999	3.07	2.93
14-Methyl-8-cholestenol	1.040	1.064	2.32	2.35
8-Cholestenol	1.050	1.070	1.91	1.95
5,25-Cholestadienol	1.070	0.998	7.18	6.82
5,24-Cholestadienol	1.090	1.084	0.50	0.52
5,7-Cholestadienol	1.090	1.087	0.24	0.25
24-Methyl-5,22-cholestadienol	1.120	1.137	1.54	1.67
7-Cholestenol	1.120	1.155	3.08	3.39
8,24-Cholestadienol	1.130	1.140	0.92	1.01
14-Methyl-7-cholestenol	1.170	1.150	1.72	1.89
24-Methyl-5-cholestenol	1.300	1.354	4.00	5.16
14,24-Dimethyl-8,24(28)-cholestadienol	1.300	1.332	2.46	3.12
24-Methyl-8,14-cholestadienol	1.330	1.337	0.56	0.71
14,24-Dimethyl-8-cholestenol	1.340	1.403	4.49	6.00
24-Methyl-8-cholestenol	1.360	1.358	0.11	0.14
4,4,14-Trimethyl-8-cholestenol	1.410	1.434	1.67	2.28
24-Methyl-5,7-cholestadienol	1.420	1.425	0.39	0.53
24-Ethyl-5,22-cholestadienol	1.420	1.442	1.59	2.18
24-Methyl-7,24(28)-cholestadienol	1.420	1.423	0.26	0.35
24-Methyl-7-cholestenol	1.460	1.493	2.27	3.23
24-Methyl-5,7,22-cholestatrienol	1.220	1.275	4.37	5.31
24-Methyl-7,22-cholestadienol	1.250	1.192	4.86	5.51
24-Methyl-5,24(28)-cholestadienol	1.260	1.282	1.75	2.14
24-Methyl-8(14)-cholestenol	1.280	1.206	6.06	6.96
14,24-Dimethyl-7,22-cholestadienol	1.290	1.338	3.63	4.62
4,24-Dimethyl-8,14-cholestadienol	1.500	1.521	1.42	2.06
14,24-Dimethyl-7-cholestenol	1.520	1.488	2.11	3.00
24-Ethyl-5,7,22-cholestatrienol	1.540	1.515	1.58	2.28
4,24-Dimethyl-8-cholestenol	1.550	1.593	2.71	4.11
24-Ethyl-5,25-cholestadienol	1.550	1.576	1.65	2.48
24-Ethyl-7,22,25-cholestatrienol	1.570	1.567	0.13	0.20
24-Ethyl-7,22-cholestadienol	1.580	1.584	0.26	0.40
24-Methyl-8,24(28)-cholestadienol	1.330	1.258	5.64	6.76
24-Ethyl-5-cholestenol	1.630	1.592	2.33	3.54
24-Ethyl-8,14-cholestadienol	1.660	1.655	0.27	0.42
24-Ethyl cholestanol	1.670	1.663	0.41	0.66
14-Methyl-24-ethyl-8-cholestenol	1.670	1.643	1.63	2.55
24-Ethyl-8-cholestenol	1.700	1.648	3.09	4.86
24-Ethyl-7,25-cholestadienol	1.730	1.777	2.67	4.53
4,14-Dimethyl-24-ethyl-8-cholestenol	1.880	1.855	1.32	2.33
4-Methyl-24-ethyl-8-cholestenol	1.920	1.833	4.72	8.25

Mean absolute error	0.03
Mean relative error	2.31
Mean error to range	2.88
Regression coefficient	0.9892
t (student) of *R*	38.2123
R signification	99.99
R square	0.9785
F(13, 45)	112.3217
F signification	99.99
Exner's function	0.094

Fig. 5. Computer outprint giving *RT* and *CT* values and relative errors for each sterol acetate.

were used to calculate the coefficients in eqn. 1 by means of a multilinear correlation technique[24–26], and these coefficients are given in Fig. 4. By means of eqn. 1, values of the retention index (*CI*) were calculated and compared with the literature data (*RI*) (see Fig. 4). A correlation coefficient of the greatest significance was obtained in all instances (>99.99 %), and the errors were acceptable from the chromatographic point of view (*ca.* 3%) for most of the compounds.

Structure (j) ——————————→ Del Re method[12–15] ——————→ E_j, $\{Q_i\}_j$
For a chemical family → $\{RI_j; E_j, \{Q_i\}_j\}$ → Multilinear correlation[24–26] → a, b, c_i
For another structure, X, belonging to this chemical family

$$RI_X = aE_X + \sum_{i=1}^{n} Q_{iX}\, c_i + b$$

Fig. 6. Scheme of calculation method.

Some molecules were eliminated from the first correlation (marked with asterisks in Fig. 4) as they gave a greater error than the mean residual deviation of the distribution. The coefficients in Fig. 4 were obtained from a second correlation, in which these molecules were excluded, but their values have been included in the figure after re-calculation. No relationship between the structures of these compounds and their anomalous behaviour was found. Fig. 4 gives the values of the coefficients for each phase, as well as their significance.

The changes in the significance, and also the value of the coefficients for each stationary phase, may be the starting point for suggesting a model of an electrostatic type in order to describe the interactions of the molecules with the stationary phase.

Fig. 4 also gives the absolute mean error, relative mean error and mean error with respect to the range, for each distribution, as well as the regression coefficients obtained, the total percentage variance (R^2), the values of t, F and its significance, and the value of the Exner function.

This system has been applied, with analogous results (see Fig. 5), to the prediction of *TRR*, d*I*, *SN*, *ΔTRR* and changes in the coefficients, but not their significance. In the case of *TRR*, for SE-30 a mean absolute error of 0.03 unit was obtained.

ABBREVIATIONS

CI	Retention index calculated from eqn. 1.
CT	Calculated retention time relative to cholesterol acetate, from eqn. 1.
d*I*	Difference between index values of two compounds on the same column.
E	Total molecular energy.
EM	Percentage relative error of calculated retention index related to Patterson's experimental index range.
ER	Relative error (%).
F	Fisher's function.
LCAO–MO	Linear combination of atomic orbitals–molecular orbitals.
Q_i	Localized charge on the ith atom.
R	Regression coefficient.

RI	Retention index according to Patterson[23].
RT	Retention time relative to cholesterol acetate according to Patterson[23].
SN	Steroid number.
t	Student's function.
TRR	Relative retention time, isothermal.

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