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Modeling of Thin-Layer Chromatographic Separation of Androstane Isomers

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Key Words

Androstane isomers
Separation modeling
Thin-layer chromatography
Mobile-phase optimization

Summary

Modeling of thin-layer chromatographic separation of androstane isomers to find the optimum mobile phase is described in this paper. The isomers of androstane are present in a variety of samples, so achieving their optimum thin-layer chromatographic separation is very important. A mathematical model was developed and tested. The model takes into account the interaction between solvents and uses a complex function for modeling, so it provides reliable results. The proposed mathematical model gives results similar to those obtained by use of other optimization models, for example the 'Simplex' and 'Prisma' methods.

1 Introduction

Androstane isomers are anabolic and androgenic steroids related to male sex hormones – 'anabolic' refers to muscle-building and 'androgenic' to increased masculine characteristics. Abuse of anabolic steroids can lead to serious health problems, some of which are irreversible. The importance of steroid analysis is apparent from many papers and book chapters [1–5].

Steroids and their metabolites are present in a variety of samples, for example biological samples and plants and pharmaceutical formulations. Modeling of thin-layer chromatography (TLC) to achieve the optimum separation is therefore very important. This optimization depends on proper choice of chromatographic conditions, especially mobile-phase composition. Many methods have been used for optimization of the mobile phase in both one and two-dimensional TLC, for example the 'Simplex' [6] and Prisma methods [7]. Computer-assisted modeling of chromatographic separation has become increasingly attractive and many software packages have been developed [8–10].

The objective of the work discussed in this paper was the modeling of thin-layer chromatographic separation of androstane isomers to find the optimum mobile-phase.

2 Experimental

Methanolic solutions (0.1%) of 5α-androstan-3β-ol (**1**), 5α-androstan-3α-ol (**2**), 5α-androstan-17β-ol (**3**), 5β-androstan-3α,17β-diol (**4**), and 5α-androstan-3β,17β-ol (**5**) (**Figure 1**) were analyzed by thin-layer chromatography on 5 cm × 10 cm silica gel 60 plates (Merck). The plates were developed at room temperature in a saturated N-chamber, by the ascending technique, using different mixtures of chloroform, acetone, and petroleum ether as moble phases (**Table 1**). The compounds were detected by spraying the dried plates with 5% ammonium molybdate and 5% sulfuric acid in water [3] then heating at 80°C; the compounds appeared as dark blue spots on a light blue background.

The plates were scanned by use of a CanoScan-Lide20 flatbed scanner with 600 × 1200 dpi resolution. The images obtained

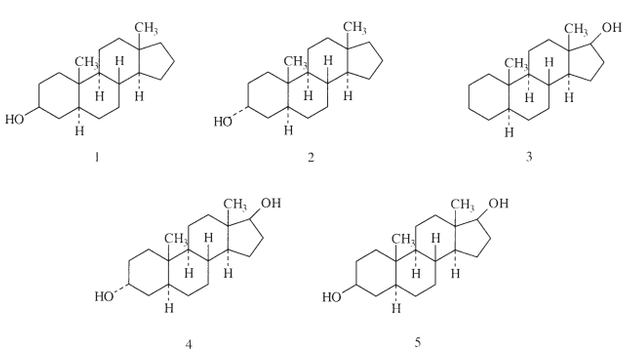

Figure 1

The structural formulas of the androstane isomers: 1, 5α-androstan-3β-ol; 2, 5α-androstan-3α-ol; 3, 5α-androstan-17β-ol; 4, 5β-androstan-3α,17β-diol; 5, 5β-androstan-3β,17β-diol.

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Table 1

The composition of the mobile phases used.

System	Chloroform [mL]	Acetone [mL]	Petroleum ether [mL]
1	10	10	10
2	0	0	10
3	0	10	0
4	10	0	0
5	50	0	50
6	50	50	0
7	0	50	50
8	10	10	80
9	80	10	10
10	10	80	10

were processed by use of computer software which selected the zone of interest from the plate image. The densitogram was extracted in three spectral fields – red, green, and blue – and the chromatogram was obtained by smoothing and baseline subtraction.

3 Results and Discussion

A mathematical model was developed and tested for modeling of the chromatographic separation of androstane isomers (Figure 1). We have previously [11] compared the classical 'Prisma' and 'Simplex' models frequently used for optimization of the mobile phase in TLC. These methods were also used for the optimization of the separation of the androstane isomers. These previous results were used for the validation of the model proposed in the work discussed in this paper.

3.1 Mathematical Model

The mathematical model correlated the experimental results, obtained by TLC, by use of different mobile phases, with an index of chromatographic performance. The model assumes that for a mixture of three solvents the index depends on mobile phase composition in accordance with the equation:

$$M7(x_1,x_2,x_3) = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + + a_7x_1x_2x_3 \quad (1)$$

where x_1, x_2, and x_3 are the molar fractions of the solvents, $M7$ is the index of chromatographic performance, and a_1, ..., a_7 are coefficients.

First, in the estimation step, the coefficients were determined by use of a procedure which minimized the sum of squared error, which provides the best estimate of the index. Then, in the prediction step, the coefficients were used to predict the index for mobile phases of any composition.

The indexes in eqs (2)–(9) were modeled by use of eq. (1):

$$R_F = R_F(i, e) = l(i)/l(e) \quad (2)$$

$$R_S(i, j, e) = 2(l(i) - l(j))/ (w(i) + w(j)) \quad (3)$$

$$R_{S0}(i, e) = 2(l_0(i) - l_0(i + 1))/(w_0(i) + w_0(i + 1)) \quad (4)$$

$$IP(e) = \sqrt{\Sigma_j(\Delta R_{F,t} - \Delta R_F(j, e))^2/n(n + 1)} \quad (5)$$

$$R_{Sa}(e) = \Sigma_j R_{S0}(j, e)/(n - 1) \quad (6)$$

$$RRP(e) = \Pi_j R_{S0}(j, e)/R_{Sa}(e) \quad (7)$$

$$Inf(e, m) = \Sigma_k(n_k/n)\log_2(n_k/n) \quad (8)$$

$$F_{ob}(e, m) = \Sigma_j a_j F_j(IP(e), \text{Inf}(e, m), R_{Sa}(e), RRP(e)) \quad (9)$$

where i and j are the compounds separated by use of mobile phase e, $l(i)$ is the migration distance of the compound, $l(e)$ is the migration distance of the mobile phase, R_F is the retention factor of compound i, $w(i)$ and $w(j)$ are zones widths, and $R_S(i, j, e)$ is the matrix of resolution values, $l_0(i)$ is the ith migration coordinate in the ordered list of migration distances, w_0 is the corresponding width, $R_{S0}(i, e)$ is the matrix of resolution values for adjacent spots, n is the number of compounds, $\Delta R_{F,t}$ is the theoretical difference between two retention factors, $\Delta R_F(j, e)$ is the jth difference between retention factors for adjacent spots, IP is the performance index, R_{Sa} is the average resolution, RRP is the relative resolution product, n_k is the number of compounds which migrate in the kth equidistant interval from the total number of intervals, m, which was divided by the whole migration distance, Inf is a quality factor computed by the *Logit* method and is zero for an ideal separation, F_j is a composite function of all four chromatographic terms ($IP(e)$, $Inf(e$, m), $R_{Sa}(e)$, and $RRP(e)$), a_j are coefficients chosen by a mathematically defined weighted relationship, and F_{ob} is an objective function which characterizes the separation with mobile phase e according to the choice of a_j coefficients, F_j functions, and the number of equidistant intervals, m.

A matrix, $\mathbf{M}_{ob}$ results from application of eqs. (2)–(9) for an array of p experiments. $\mathbf{M}_{ob}$ has one or more rows and, always, p columns, one for every experiment; its elements represent the values of the index modeled.

To provide a unique solution by use of the optimization algorithm the imposed prerequisite of eq. (1) is $p \geq 7$.

3.2 Optimization Procedure

A system of p linear equations with seven terms is constructed for every row of the matrix $\mathbf{M}_{ob}$ on the basis of eq. (1). The partial least-squares method is applied to the system of linear equations to obtain the coefficients. The *Gauss* method is then used for system solving, and the solution is $A_0 = (a_{01}, ..., a_{07})$. The values determined for the coefficients are used to predict the chromatographic index by use of eq. (1).

If the chromatographic index is Y, and z is the number of rows of matrix $\mathbf{M}_{ob}$ and, consequently, the number of predictors, the estimate of the chromatographic index, $\hat{Y}$, can be expressed as an array:

$$\hat{Y} = (\hat{y}_1, \ldots \hat{y}_z) \quad (10)$$

The optimum of $\hat{Y}$ can be obtained by applying of function that tends to a maximum or to a minimum in the optimum case, depending on the type of Y:

$$\hat{Y}_0 = \text{opt}(\hat{Y}) \quad (11)$$

where opt = 'max' or 'min'. The optimum point, i.e. the optimum mobile phase composition, can be obtained by examining the entire domain of possible natural values of mobile phase composition.

Table 2

Migration distance [cm] and spot width [cm] for the compounds analyzed.

System	L	l_1	w_1	l_2	w_2	l_3	w_3	l_4	w_4	l_5	w_5
1	8.70	6.65	0.48	7.36	0.35	7.26	0.23	4.00	0.38	4.76	0.98
2	8.83	0.00	0.42	0.00	0.44	0.00	0.22	0.00	0.25	0.00	0.21
3	8.75	8.29	0.37	8.49	0.26	8.49	0.11	7.93	0.28	7.79	0.59
4	9.00	1.21	0.62	2.05	0.45	1.43	0.41	0.05	0.23	0.19	0.30
5	8.93	0.54	0.56	0.98	0.38	0.68	0.27	0.00	0.26	0.00	0.25
6	8.84	6.71	0.55	7.12	0.31	7.05	0.20	5.31	0.36	5.56	0.69
7	8.76	8.44	0.36	8.56	0.11	8.56	0.05	7.35	0.31	7.20	1.38
8	8.86	3.49	0.60	4.71	0.42	4.51	0.28	0.53	0.27	0.64	1.41
9	8.87	5.08	0.69	6.71	0.51	6.06	0.34	1.01	0.32	2.32	0.63
10	8.82	8.24	0.52	8.41	0.24	8.46	0.14	7.38	0.32	7.27	0.96

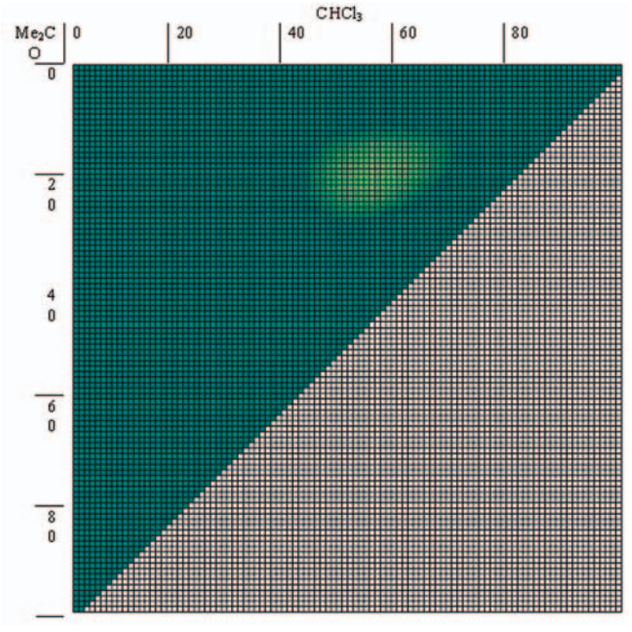

Figure 2

The objective function diagram.

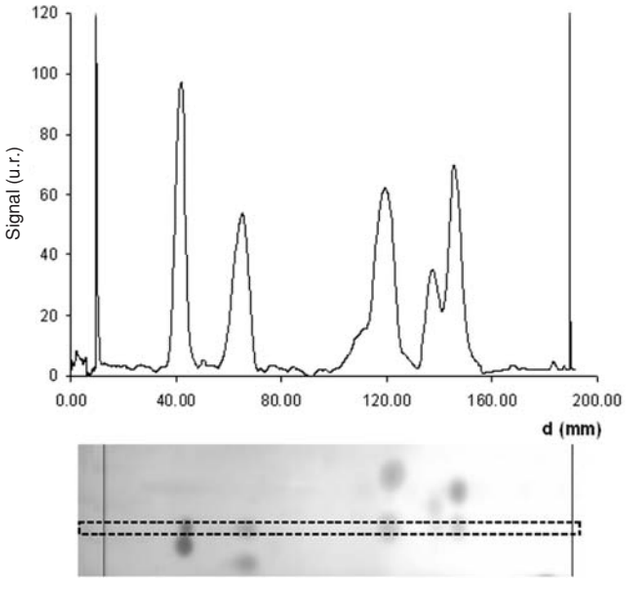

Figure 3

The chromatographic separation achieved by elution with the optimum mobile phase.

4 Experimental Application

In this part the software was implemented in accordance with the model specifications. Ten chromatographic runs were performed using mobile phases of different composition (Table 1). The experimental data (**Table 2**) were used as input to the software to obtain the objective function diagram (**Figure 2**). From this figure it can be seen that the optimum mobile phase composition is chloroform–acetone–petroleum ether, 55 + 19 + 26 (*v*/*v*).

The optimum composition obtained with the model was tested experimentally; the separation obtained is shown in **Figure 3**. It is apparent from Figure 3 that good separation of all the compounds is achieved. This separation is, moreover, very similar to those obtained by elution with mobile phases optimized by use of the 'Simplex' and 'Prisma' methods [11], which confirms the validity of the model. It can, moreover, be concluded that for these moderately polar compounds small modifications of mobile phase composition do not lead to substantial changes of compound retention.

5 Conclusion

Modeling of chromatographic separation is very useful for optimization of mobile phase composition. The proposed mathematical model takes into consideration the interactions between solvents and uses a complex function for modeling, so it provides reliable results. The proposed mathematical model gives results similar to those obtained by use of other optimization models. The advantage of this model is that on the basis of only 7–10 chromatographic runs chromatographic separations for all mobile phase compositions can be predicted.

References

[1] *M. Liu, L.-S. Li, S.-L. Da*, and *Y.-Q. Feng*, Talanta **66** (2005) 479–486.

[2] *M. Szumski* and *B. Buszewski*, J. Sep. Sci. **27** (2004) 837–842.

[3] *J. Sherma* and *B. Fried*, Handbook of Thin Layer Chromatography, Marcel Dekker, New York, USA, 1996.

[4] *M. Mulja* and *G. Indrayanto*, in: Encyclopedia of Chromatography, Marcel Dekker, New York, USA, 2001.

[5] *J. Sherma*, in: *J. Sherma* and *B. Fried* (Eds) Handbook of Thin Layer Chromatography, Marcel Dekker, New York, USA, 2003.

[6] *L.G. Sabate* and *X. Tomas*, J. High Resolut. Chromatogr. Chromatogr. Commun. **7** (1984) 104–106.

[7] *Sz. Nyiredy, B. Meier, C.A.J. Erdelmeier*, and *O. Sticher*, J. High Resolut. Chromatogr. Chromatogr. Commun. **8** (1985) 186–192.

[8] *C. Cimpoiu*, in: *J. Sherma* and *B. Fried* (Eds) Handbook of Thin Layer Chromatography, Marcel Dekker, New York, USA, 2003.

[9] *S. Heinisch, J-L. Rocca*, and *M. Feinberg*, J. Chemom. **3** (1989) 127–137.

[10] *C. Cimpoiu, L. Jäntschi*, and *T. Hodisan*, J. Liq. Chromatogr. Related Technol. **22** (1999) 1429–1441.

[11] *C. Cimpoiu, A. Hosu*, and *S. Hodisan*, J. Pharm. Biomed. Anal. **39** (2005) 1872–1877.

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