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Analysis of Some Steroids by TLC Using Optimum Mobile Phases

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Abstract

The paper presents a separation study of some steroids isomers by TLC. Optimization of the mobile phase was achieved using ”Prisma”, “ Simplex “ and mathematical models. These models are simple, rapid methods for optimization and many mobile phase compositions can be evaluated simultaneously.

Keywords: Prisma, Simplex, Optimization, Steroids.

I. Introduction

Steroids are natural (figure 1.) or synthetic compounds with a cyanopentanoperhydrophenanthezene skeleton and include bile acids, androgens, estrogens, corticosteroids, sterols and vitamin D.

The different classes of steroids differ by the nature of their substitutions, by the degree of unsaturation of the tetracyclic nucleus as well as by the nature of side- chains R_1, R_2. When the radical R_2 from the position 17 is hydrogen, a compound class named androstans can be obtained. We have studied the possibility of separation of some

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pharmaceutically active isomers. The isomers have almost the same structures, differing by the number and position of hydroxyl radical. The choice of proper mobile phase and the optimization of the mobile phase composition are very important, because chromatographic separation is difficult to achieve [1]

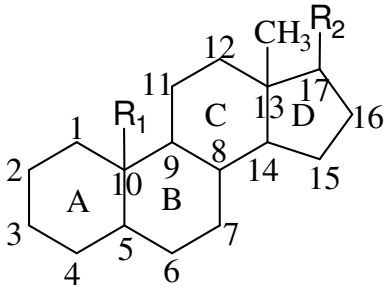


Fig. 1. The general formula of the steroids.

In a previous article [2] the results obtained at the separation of these isomers, using optimum mobile phase, were published. The mobile phase system (chloroform: acetone: petroleum ether) was selected from seven different systems presented in the literature [3, 4]. The other have been conceived by us, through the method of numerical taxonomy. This optimization has been achieved through the Simplex and Prisma methods. Comparing the chromatographic results obtained by elution with optimum mobile phases, it can be concluded that they do not differ significantly and both mobile phases have a very similar efficiency. Later, a mathematical model has been conceived that correlates the experimental results obtained by a restrained number of the mobile phase compositions with any possible composition of this one [5].

In this work the optimizing program was tested for another mobile phase system, by comparing with the classical models (Simplex and Prisma).

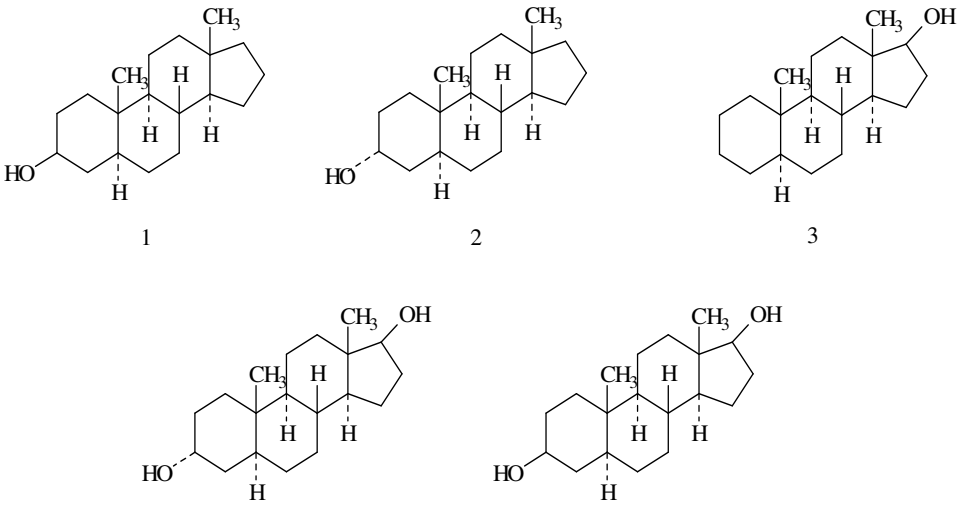
II. Experimental

The chosen mobile phase system contains, besides chloroform, solvents from the same group of selectivity (the eight known) but of different strength as compared with the tested solvents from the previous work: chloroform - ethyl methyl ketone - cyclohexane.

The chromatographic plates with silica gel 60 F254 (Merck) were developed at room temperature in a saturated N-chamber, by the ascending technique.

The solutions (0,1%) of the compounds (figure 2) were prepared in methanol.

The detection was made by spraying with 5% ammonium molibdate and sulfuric acid in water and heated at 80^{o}C. The components appear as dark blue spots.



1 2 3

4 5

Fig. 2. 1 – 5α androstane 3β-ol; 2 – 5α androstane 3α-ol; 3 – 5α androstane 17β-ol; 4 – 5β androstane 3α, 17β-diol; 5 – 5β androstane 3β,17β-diol

III. Results and discussions

The quality of the chromatographic separation was estimated by means of the objective function F_{obj}, which take a minimum value for the optimum separation.

$$F_{obj} = a \cdot I_p + b/I + c/I \cdot \overline{R_S} + d/RRP \qquad (1)$$

where: a, b, c, d are arbitrary weighting coefficients; I_p - performance index [4] (eq. 2); I – amount of information [5] (eq. 3); $\overline{R_S}$ - medium resolution; RRP-relative resolution product [6] (eq. 4);

$$I_p = \sqrt{\sum \left(\frac{\left(\Delta hR_{f,i} - \Delta hR_{f,t}\right)^2}{n(n+1)} \right)} \qquad (2)$$

$$I = - \sum (n_k/n) \lg_2 (n_k/n) \qquad (3)$$

$$RRP = \frac{\Pi R_{s,i}}{\overline{R_S}} \qquad (4)$$

"Simplex" method

First, three compositions of the mobile phase (table 1) were chosen so as to form an equilateral triangle in the variable space.

The CRF is evaluated in each vertex of the figure 3, the most unfavorable vertex (point 1) corresponding to the worst response is rejected and then a new favorable vertex (point 4) is established by searching the direction that is experienced by this unfavorable vertex and the centroid of the other vertices. The new simplex is thus determined and the algorithm is repeated until the optimum response is obtained.

Table 1. The mobile phase composition and the corresponding values of F_{obj} and the Simplex generation.

No	Chloroform – Cyclohexane – MEK (v/v/v)	F_{obj}	Simplex
1	3 : 2.5 : 14.5	33.66	1 – 2 – 3
2	3 : 5.5 : 11.5	22.84	
3	5.6 : 4 : 10.4	20,61	
4	5.6 : 7 : 7.4	26.00	2 – 3 – 4
5	4.95 : 5.9 : 9.15	21.00	2 – 3 – 5
6	7.5 : 4.6 : 7.9	20.35	3 – 5 – 6
7	8.3 : 2.9 : 8.8	23.15	3 – 6 – 7
8	7.5 : 3.5 : 9	20.50	3 – 6 – 8
9	9.5 : 4.1 : 6.7	17.57	6 – 8 – 9
10	9.5 : 5 : 5.5	16.06	6 – 9 – 10
11	11.5 : 4.6 : 3.9	15.64	9 – 10 – 11
12	11.5 : 5.6 : 2.9	14.9	10 – 11 – 12
13	10.5 : 5.1 : 4.4	13.2	11 – 12 – 13

The optimum composition that was found by the evaluation of the separation according to F_{obj} corresponds to point 13. The optimum composition of the tested mobile phase system presented in table 1 is chloroform : cyclohexane : MEK (10.5 : 5.1 : 4.4 v/v). The separation obtained by elution with optimum mobile phase is presented in figure 4.

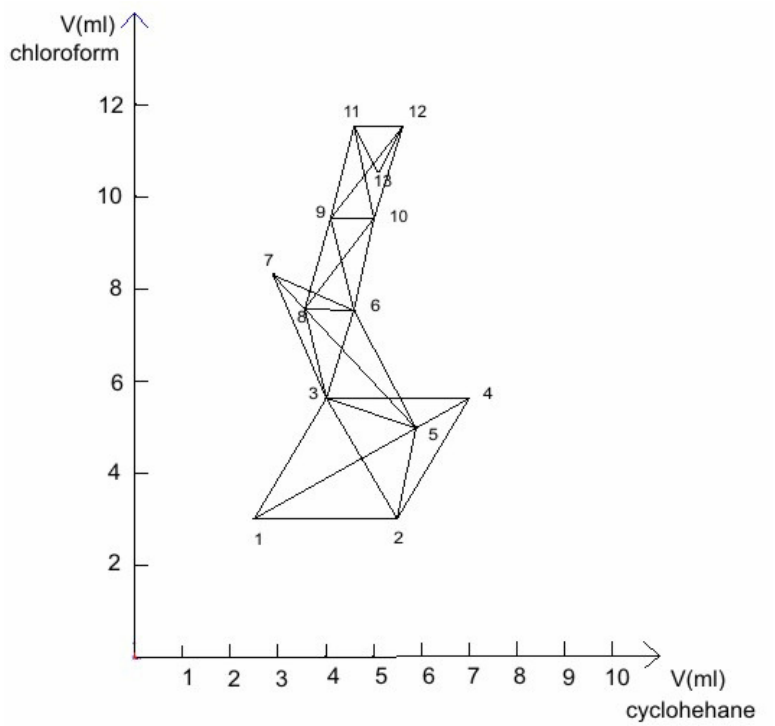


Fig. 3. Simplex model used for the optimization of the mobile phase.

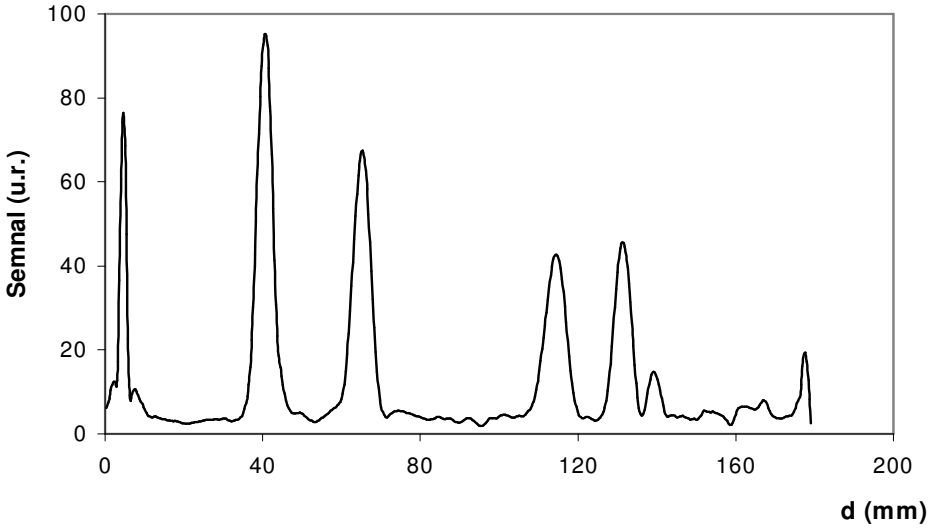


Fig. 4. The chromatographic separation performed with optimum mobile phase obtained by Simplex method.

"Prisma" method

The optimization was started with the composition of mobile phase (cyclohexane : chloroform : MEK) corresponding to the center of the triangle top face of the regular part of prisma (Ps=333) and three other selectivity points close to the axes of the triangle (Ps = 811, 118 and 181). If separation was insufficient other selectivity points were tested around the solvent combination that gave the best separation. This process was repeated until the best solvent composition was obtained (figure 5).

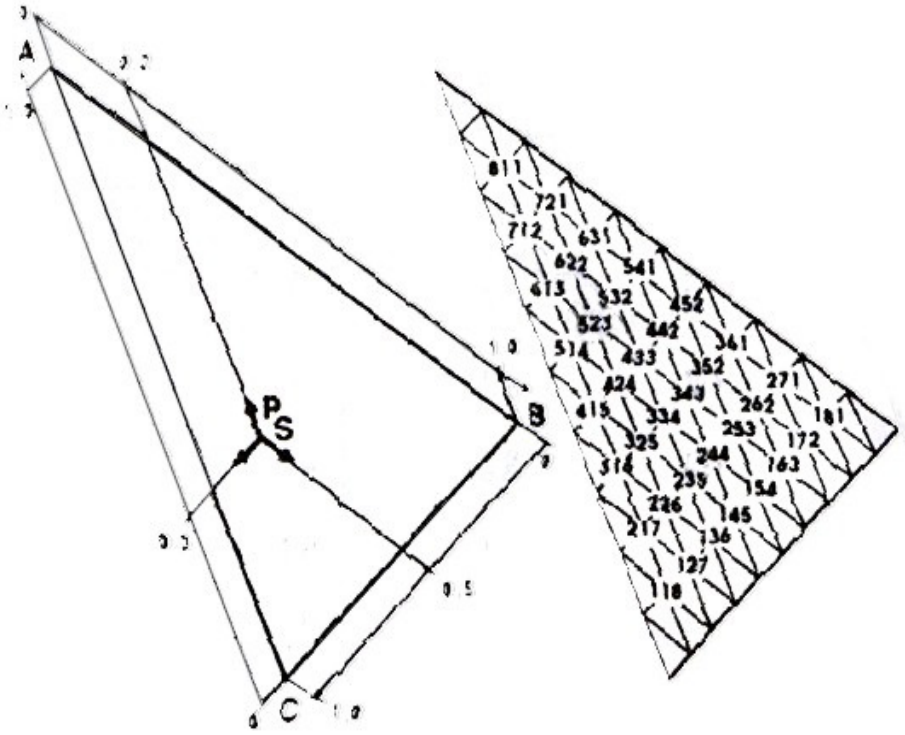


Fig. 5. Prisma model for the optimization of the mobile phase

As it can be seen from table 2, the smallest value of F_{obj} correspond to the P_S = 811. Then the other selectivity points around this point were tested. The process was repeated until the best separation is obtained.

The optimum composition of the tested mobile phase system by "Prisma" method is chloroform – cyclohexane – MEK (50 : 30 : 20) and the separation is presented in figure 6.

Table 2. The composition of mobile phases and the corresponding values of F_{obj}.

No	Chloroform – Cyclohexane – MEK (v/v)	F_{obj}
1	33 : 33 : 33	21.75
2	10 : 80 : 10	36.11
3	80 : 10 : 10	13.59
4	10 : 10 : 80	272.84
5	70 : 10 : 20	13.81
6	70 : 20 : 10	14.27
7	60 : 20 : 20	14.54
8	60 : 10 : 30	16.53
9	50 : 30 : 20	11.34

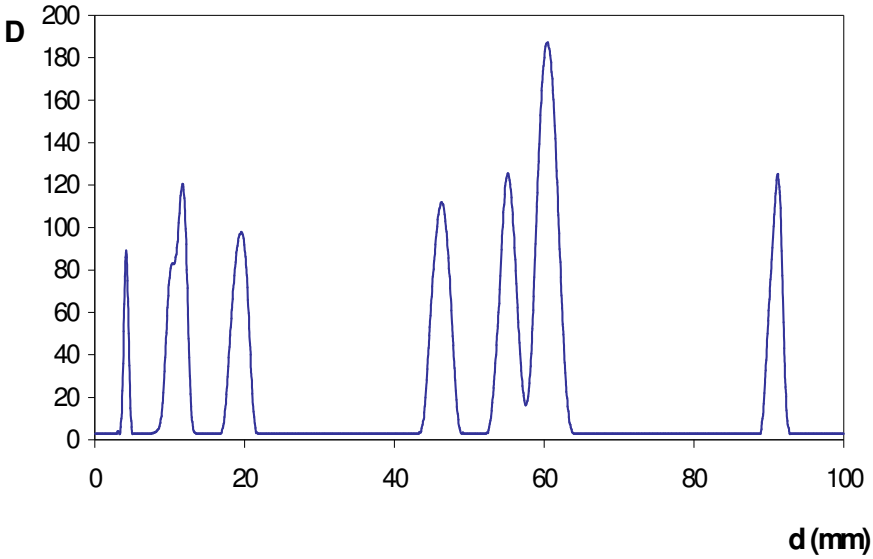


Fig. 6. The chromatographic separation performed with optimum mobile phase obtained by Prisma model.

Mathematical model

The mathematical model assumes that a program is used for the optimization of mobile phase composition. Applying the mathematical model to the experimental data presented in the table 3, the diagram of the objective function presented in figure 7 was obtained. It can be seen from figure 7 that an optimum region exists (the light region). In this region the optimum composition of mobile phase system can be established according to the F_{obj} value. The optimum mobile phase composition is chloroform – cyclohexane –

MEK 54 : 18 : 28, v/v and the optimum chromatographic separation is presented in figure 8.

Table 3. Experimental data used for the optimization of mobile phase composition by mathematical model.

System	I_1	w_1	I_2	w_2	I_3	w_3	I_4	w_4	I_5	w_5
1 : 1 : 1 L = 7.76	5.51	0.50	6.1	0.31	6.09	0.19	2.45	0.26	3.32	0.64
2 : 16 : 2 L = 9.64	2.05	0.27	2.97	0.21	2.76	0.22	0.15	0.23	0.28	0.51
20 : 0 : 0 L = 8.77	0.92	0.43	1.89	0.32	2.47	0.21	0.24	0.22	0.26	0.22
10 : 10 : 0 L = 9.04	0.53	0.41	0.96	0.31	0.75	0.22	0.15	0.25	0.2	0.25
2 : 2 : 16 L = 8.84	7.43	0.51	7.92	0.21	7.91	0.19	6.14	0.31	6.48	0.32
16 : 2 : 2 L = 8.91	3.45	0.54	5.03	0.31	4.59	0.29	0.58	0.21	1.33	0.35
0 : 20 : 0 L = 8.41	0.00	0.46	0.00	0.32	0.00	0.15	0.00	0.24	0.00	0.21
0 : 0 : 20 L = 8.93	8.28	0.31	8.42	0.16	8.34	0.15	7.29	0.21	7.00	0.47
10 : 0 : 10 L = 8.85	6.23	0.56	0.85	0.31	6.89	0.24	4	0.29	4.82	0.51
0 : 10 : 10 L = 8.55	6.44	0.52	6.83	0.21	6.8	0.11	5.64	0.21	4.1	0.82

Comparing the separations obtained with the optimized mobile phase composition by the three above mentioned methods, it can be concluded that these separations do not differ significantly from the point of view of their efficiency.

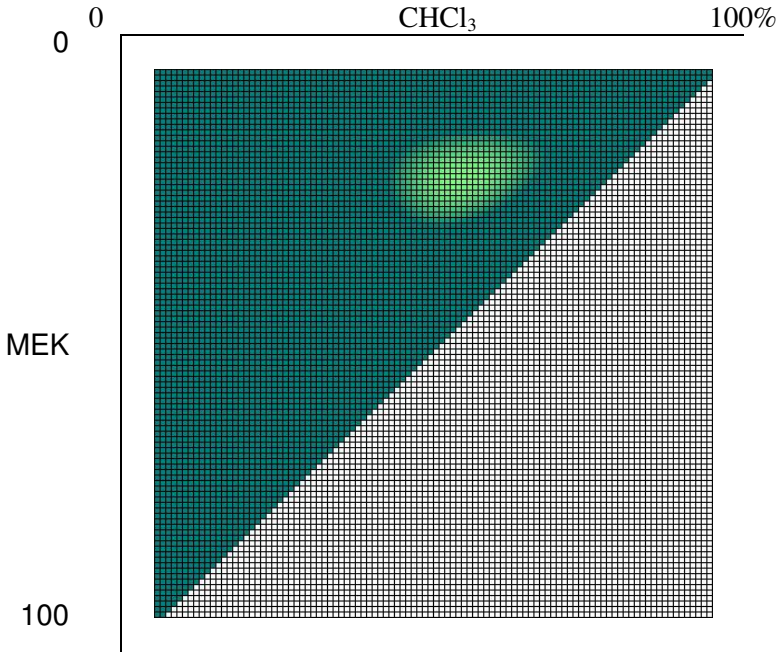


Fig.8. The diagram of the objective function.

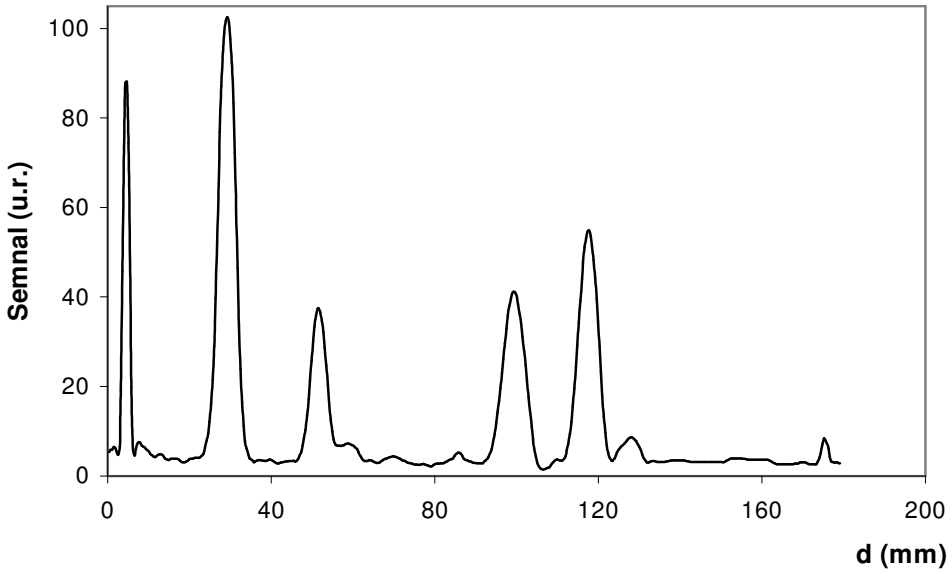


Fig. 7. The chromatographic separation performed with optimum mobile phase obtained by mathematical model.

IV. Conclusions

The "Simplex", "Prisma" and mathematical model are simple and rapid methods for optimization and many mobile phase compositions can be evaluated simultaneously.

Using the optimum mobile phase, all androstane isomers can be separated from mixture even if they are similar structures.

The advantage of these optimization methods is that optimum composition of mobile phase can be easily obtained.

V. References

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