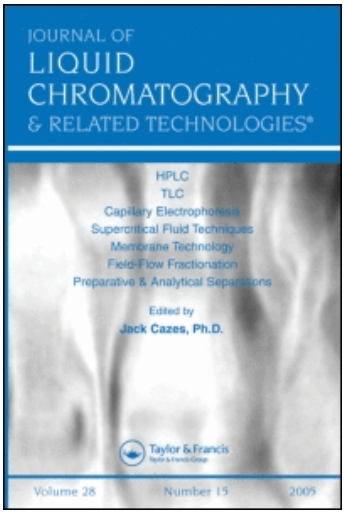

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Influence of Temperature of Silica Gel Activation on Separation of Selected Biologically Active Steroid Compounds

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Influence of Temperature of Silica Gel Activation on Separation of Selected Biologically Active Steroid Compounds

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Abstract: Selected steroid compounds (androsterone, epi-androsterone, dehydro-epi-androsterone, testosterone, stigmasterol, β-sitosterol, estradiol, hydrocortisone, and cholesterol) were separated by adsorption TLC on silica gel 60, not activated, and activated at temperatures of 100°C, 120°C, 150°C, and 200°C, during 15, 30, 60, and 120 min, respectively. The mixture of chloroform and acetone (85 : 15, v/v) was used as mobile phase. The lowest R_F values of particular substances investigated were obtained on chromatographic plates precoated by silica gel activated at a temperature of 120°C. The time and activation temperature of silica gel influenced the order of substances adsorbed, the values of separation factors: ΔR_F, R_F^{α} and α. None of the chromatographic conditions used allowed for the separation of the following pairs of substances: androsterone-dehydro-epi-androsterone, and testosterone-estradiol.

Keywords: TLC, Separation, Steroid compounds, Drugs, Activation of silica gel, Temperature

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INTRODUCTION

Steroids are compounds having a four-ringed carbon skeleton derived from 1,2-cyclopentanoperhydrophenanthrene. Many steroids are present in plants and animals. Cholesterol is the most important sterol representative of animal origin. A series of cholesterol analogs has been isolated from many organisms. For example, stigmasterol is present in soya-bean oil, along with sitosterol; these sterols occur in many other plant oils, and in beetroots, celery, and other vegetables. Stigmasterol is involved in phosphate (V) metabolism and is the initial product in the semisynthesis of steroid hormones. β-Sitosterol is present mainly in plants. From 55 to 75% of the sterol fraction of plant oils is β-sitosterol. Both β-sitosterol and stigmasterol are important in the semisynthesis of steroid hormones.[1,2] Sex hormones and adrenocortical hormones are the most important steroid hormones. Sex hormones are divided into two groups: the male and female sex hormones.[1,2] They have definite physiological activities.[3–10] Some steroid compounds, i.e., testosterone, estradiol, estriol, progesterone, hydrocortisone, and β-sitosterol are used as drugs in modern therapy.

Plates can be activated by temperature, chemical reagents, or by impregnation. Among these parameters, temperature causes a change in the activity of the silica gel surface. Because of this, investigations concerning the influence of temperature on the change of activity of silica gel were used to separate selected steroid compounds.

EXPERIMENTAL

Chemicals

The following components of the mobile phase chloroform (POCh, Gliwice, Poland) and acetone (Chempur, Piekary Śląskie, Poland) were used for adsorption TLC analysis. The commercial samples of androsterone (A), epi-androsterone (EP), dehydro-epi-androsterone (DHEA), testosterone (T), stigmasterol (ST), β-sitosterol (S), estradiol (E), hydrocortisone (H), and cholesterol (CH) (E. Merck, Germany) were used as test solutes. Methanol (POCh, Gliwice, Poland; pure p. a.), ethanol (ZPS Polmos, Kutno, Poland), chloroform (POCh, Gliwice, Poland) and acetone (Chempur, Piekary Śląskie, Poland) were used to prepare the solutions of steroid compounds. Sulfuric acid, 95% (Chempur, Piekary Śląskie, Poland) and methanol (POCh, Gliwice, Poland) were used to prepare the visualizing reagent.

Thin-Layer Chromatography

TLC was performed on 20 × 20 cm aluminium foil-backed plates precoated with 0.2 mm layer of a silica gel 60 (E. Merck, #1.05553). The plates were

activated at 100°C, 120°C, 150°C, and 200°C for 15, 30, 60, and 120 min, respectively. Standard solutions of steroid compounds (5 mg/1 mL) were prepared in methanol (for androsterone, epi-androsterone, and estradiol, cholesterol), chloroform (for dehydro-epi-androsterone, stigmasterol, and β-sitosterol), ethanol (for testosterone), or a mixture chloroform and acetone ($7 + 3$, *v/v;* for hydrocortisone). Solutions of the standards, estradiol and hydrocortisone, were spotted onto a chromatographic plate in 5 μL quantities, and the remaining compounds in 1 μL quantities. The particular compounds were spotted separately onto the plates. The steroid compounds were separated using chloroform + acetone (85 : 15, v/v) as mobile phase. Fifty mL of mobile phase was placed in a conventional chromatographic tank (Camag, Switzerland). The tank was saturated with solvent for 20 min. The chromatograms were developed at the room temperature, e.g., 22°C. The development distance was 14 cm. The plates were dried at room temperature, i.e., 22°C. A mixture of sulfuric acid and methanol (1:9, v/v) was used as the visualizing agent and a 20 cm × 20 cm plate was sprayed with 10 mL of this visualizing agent. The plate was then heated at 120°C for 15 min. The chromatograms were done in triplicate and mean R_F values were calculated.

Separation Parameters

Separation factor (α),[11] constant of the pair separation (R_F^{α}),[12] and ΔR_F were calculated for all the chromatograms.

The separation factor (α) was calculated using the equation:

$$\alpha = \frac{(1/R_{F1}) - 1}{(1/R_{F2}) - 1} \tag{1}$$

where: R_{F1} and R_{F2} are values of R_F of two adjacent spots on the chromatograms, and $R_{F1} < R_{F2}$.

The constant of the pair separation (R_F^{α}) was calculated for the investigated compounds as the ratio of the R_F values of the two adjacent spots on the chromatogram:

$$R^{\alpha}_{F(1,2)} = \frac{R_{F1}}{R_{F2}} \tag{2}$$

where: R_{F1} and R_{F2} are the values of the two adjacent spots; and $R_{F1} > R_{F2}$.

ΔR_F was calculated according to the formula:

$$\Delta R_{F(1,2)} = R_{F1} - R_{F2} \tag{3}$$

where: R_{F1} and R_{F2} are the values of the two adjacent spots; and $R_{F1} > R_{F2}$.

RESULTS AND DISCUSSION

Comparisons of R_F values of steroid compounds investigated on plates not activated and activated at temperatures of 100°C, 120°C, 150°C, and 180°C during 15, 30, 60, and 120 min, respectively, are presented in Figures 1–4. From the comparisons presented, it is apparent that the lowest R_F values of individual substances investigated were obtained when the chromatographic plates precoated with silica gel were activated at a temperature of 120°C, regardless of the duration of the activation. The R_F values obtained indicate that, for all the chromatographic conditions used in regard to changes of activated plates precoated with silica gel, hydrocortisone was adsorbed the strongest. The adsorption of the remaining steroid compounds investigated was different in relation to temperature and time of activation of the chromatographic the plates.

Our findings suggest that the time and temperature of silica gel activation influence the order of adsorption of the substances investigated, and the separation factor values. Two substances are completely separated if differences in the R_F values of these substances are larger than or equal to 0.05 ($\Delta R_F \geq 0.05$).[13–15]

From the mixture of nine steroid compounds investigated on plates precoated with non-activated silica gel, considering their order of separation on silica gel, they separated as follows: hydrocortisone from estradiol ($\Delta R_{F(H/E)} = 0.50$), testosterone from epi-androsterone ($\Delta R_{F(T/EP)} = 0.05$), and androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.07$).

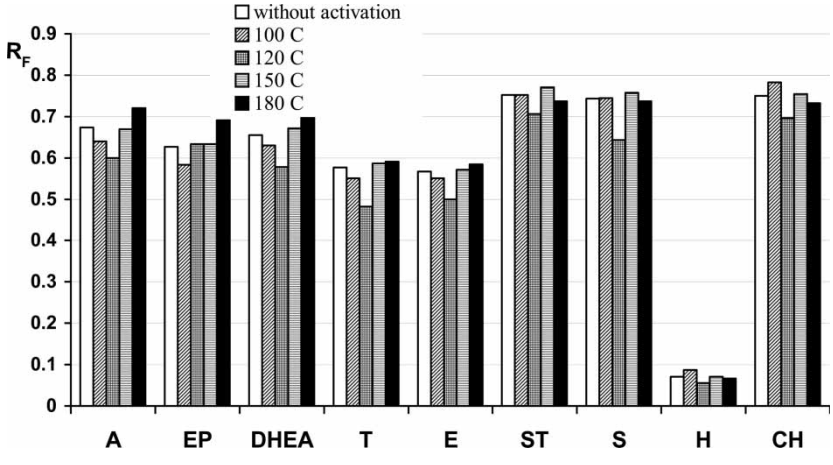

Figure 1. Comparison of R_F values of steroid compounds investigated on non-activated plates and those activated for 15 min at a temperature of 100°C, 120°C, 150°C, and 180°C. The abbreviations of steroids are explained in EXPERIMENTAL. Chemicals.

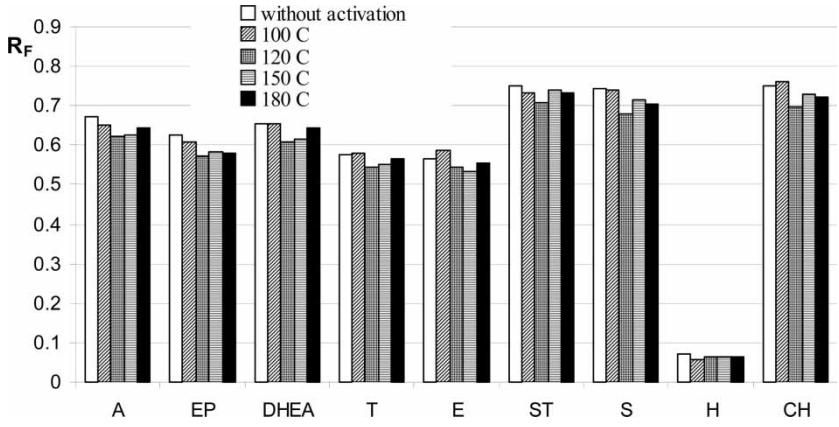

Figure 2. Comparison of R_F values of steroid compounds investigated on non-activated plates and those activated for 30 min at a temperature of 100°C, 120°C, 150°C, and 180°C. The abbreviations of steroids are explained in EXPERIMENTAL. Chemicals.

On plates precoated with silica gel activated for 15 min at a temperature of 100°C, hydrocortisone was separated from testosterone ($\Delta R_{F(H/T)} = 0.46$), androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.10$), and epi-androsterone from dehydro-epi-androsterone ($\Delta R_{F(EP/DHEA)} = 0.05$); at a temperature of 120°C, hydrocortisone was separated from testosterone ($\Delta R_{F(H/T)} = 0.43$), estradiol from dehydro-epi-androsterone ($\Delta R_{F(E/DHEA)} = 0.08$) and β-sitosterol from

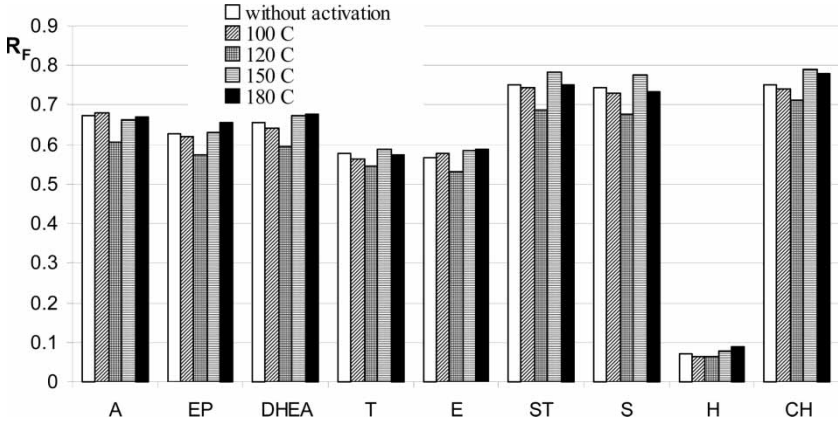

Figure 3. Comparison of R_F values of steroid compounds investigated on non-activated plates and those activated for 60 min at a temperature of 100°C, 120°C, 150°C, and 180°C. The abbreviations of steroids are explained in Part: EXPERIMENTAL. Chemicals.

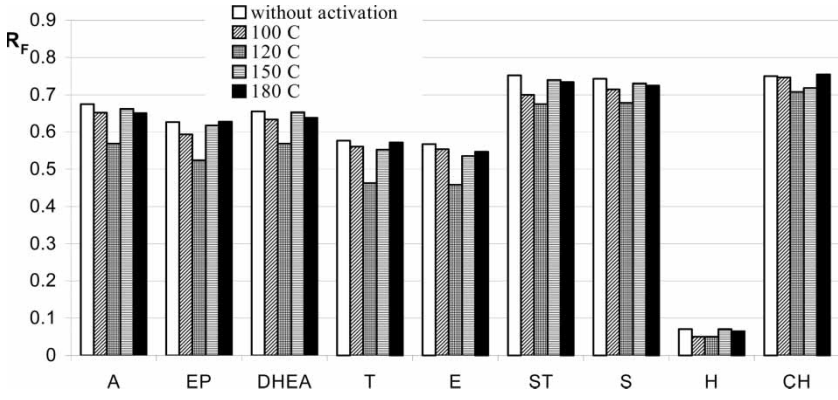

Figure 4. Comparison of R_F values of steroid compounds investigated on non-activated plates and those activated for 120 min at a temperature of 100°C, 120°C, 150°C, and 180°C. The abbreviations of steroids are explained in Part: EXPERIMENTAL. Chemicals.

cholesterol ($\Delta R_{F(S/CH)} = 0.05$); at a temperature of 150°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.50$) and dehydro-epi-androsterone from cholesterol ($\Delta R_{F(DHEA/CH)} = 0.08$); at a temperature of 180°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.52$) and testosterone from epi-androsterone ($\Delta R_{F(T/EP)} = 0.10$).

On silica gel activated for 30 min at a temperature of 100°C, hydrocortisone was separated from testosterone ($\Delta R_{F(H/T)} = 0.52$) and dehydro-epi-androsterone from stigmasterol ($\Delta R_{F(DHEA/ST)} = 0.08$); at a temperature of 120°C, hydrocortisone was separated from testosterone ($\Delta R_{F(H/T)} = 0.48$) and androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.06$); at a temperature of 150°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.47$) and androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.09$); at a temperature of 180°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.49$), epi-androsterone from androsterone ($\Delta R_{F(EP/A)} = 0.06$), and dehydro-epi-androsterone from β-sitosterol ($\Delta R_{F(DHEA/S)} = 0.06$).

On silica gel activated for 60 min at a temperature of 100°C, hydrocortisone was separated from testosterone ($\Delta R_{F(H/T)} = 0.50$) and androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.05$); at a temperature of 120°C hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.47$) and androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.06$); at a temperature of 150°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.50$) and dehydro-epi-androsterone from β-sitosterol ($\Delta R_{F(DHEA/S)} = 0.10$); at a temperature of 180°C, hydrocortisone was separated from testosterone ($\Delta R_{F(H/T)} = 0.49$) and dehydro-epi-androsterone from β-sitosterol ($\Delta R_{F(DHEA/S)} = 0.06$).

On silica gel activated for 120 min at a temperature of 100°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.50$) and stigmasterol from

β-sitosterol ($\Delta R_{F(ST/S)} = 0.05$); at a temperature of 120°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.41$), testosterone from epi-androsterone ($\Delta R_{F(T/EP)} = 0.06$) and dehydro-epi-androsterone from stigmasterol ($\Delta R_{F(DHEA/ST} = 0.10$); at a temperature of 150°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.46$), testosterone from epi-androsterone ($\Delta R_{F(T/EP)} = 0.07$) and androsterone from cholesterol ($\Delta R_{F(A/CH)} = 0.06$); at a temperature of 180°C, hydrocortisone was separated from estradiol ($\Delta R_{F(H/E)} = 0.48$), testosterone from epi-androsterone ($\Delta R_{F(T/EP)} = 0.06$) and androsterone from β-sitosterol ($\Delta R_{F(A/S)} = 0.07$).

Our findings suggest that a linear regression occurs among R_F values obtained on silica gel non-activated and activated at different times and temperatures. Nevertheless, it was stated, in some cases, that changes of silica gel activation can improve the separation of some pairs of substances investigated. For example, androsterone (A) from β-sitosterol (S) separated on non activated plates ($\Delta R_{F(A/S)} = 0.07$); however, on plates activated for 15 min at a temperature of 100°C, separation of these substances improved ($\Delta R_{F(A/S)} = 0.10$). On the other hand, β-sitosterol (S) was not separated from cholesterol (CH) on non-activated plates ($\Delta R_{F(S/CH)} = 0.01$) while, on silica gel activated plates at a temperature of 120°C for 15 min, these substances separated completely ($\Delta R_{F(S/CH)} = 0.05$).

Chromatographic conditions for the best separations of particular pairs of substances investigated are presented in Table 1. From these data, it is apparent that none of the chromatographic conditions used allow for the separation of the pairs of substances investigated as follows: androsterone from dehydro-epi-androsterone and testosterone from estradiol.

CONCLUSIONS

On the basis the research done herein, we conclude that it was not possible to separate all of the steroid compounds investigated by using only the same chromatographic conditions.

In all the chromatographic conditions used, in regard to changes of activation of plates precoated with silica gel, hydrocortisone adsorbed the most strongly. Adsorption of the remaining compounds investigated was different in relation to the temperature and the time of activation of the chromatographic plates. The lowest R_F values of individual substances investigated were obtained on chromatographic plates precoated with silica gel activated at a temperature of 120°C. The time and temperature of activation of silica gel influenced, not only the order of adsorption of substances investigated, but also the values of the separation factors: ΔR_F, R_F^{α}, and α.

None of the chromatographic conditions used allowed for the separation the following pairs of substances investigated: androsterone–dehydro-epi-androsterone and testosterone–estradiol.

Table 1. Chromatographic conditions for the best separations of particular pairs of substances investigated

Pair of steroid compounds[a]	Activation of silica gel (time, temperature)	ΔR_F	R_F^{α}	α
A/EP	30 min, 180°C	0.06	1.11	1.32
A/DHEA	60 min, 100°C	0.04	1.06	1.19
A/T	15 min, 180°C	0.13	1.22	1.78
A/E	15 min, 180°C	0.14	1.23	1.83
A/ST	60 min, 150°C	0.12	1.18	1.84
A/S	60 min, 150°C	0.11	1.17	1.76
A/H	15 min, 180°C	0.65	11.09	36.38
A/CH	15 min, 100°C	0.14	1.22	2.03
EP/DHEA	30 min, 180°C	0.06	1.11	1.32
EP/T	15 min, 120°C	0.15	1.31	1.85
EP/E	15 min, 120°C	0.13	1.27	1.72
EP/ST	30 min, 150°C	0.16	1.26	2.02
EP/S	15 min, 100°C	0.16	1.28	2.08
EP/H	15 min, 180°C	0.62	10.50	31.66
EP/CH	15 min, 100°C	0.20	1.34	2.58
DHEA/T	15 min, 180°C	0.15	1.26	2.00
DHEA/E	15 min, 180°C	0.16	1.27	2.06
DHEA/ST	15 min, 120°C	0.13	1.22	1.76
DHEA/S	15 min, 100°C	0.11	1.18	1.71
DHEA/H	15 min, 180°C	0.68	11.26	40.90
DHEA/CH	15 min, 100°C	0.15	1.24	2.12
T/E	120 min, 180°C	0.02	1.05	1.11
T/ST	120 min, 120°C	0.21	1.46	2.41
T/S	120 min, 120°C	0.22	1.47	2.45
T/H	30 min, 100°C	0.52	10.00	22.44
T/CH	120 min, 120°C	0.24	1.53	2.81
E/ST	120 min, 120°C	0.22	1.47	2.45
E/S	120 min, 120°C	0.22	1.48	2.49
E/H	30 min, 10°C	0.53	10.12	23.08
E/CH	120 min, 120°C	0.25	1.54	2.86
ST/S	15 min, 120°C	0.06	1.10	1.34
ST/H	60 min, 150°C	0.70	9.92	42.32
ST/CH	120 min, 100°C	0.05	1.07	1.26
S/H	60 min, 150°C	0.70	9.84	40.62
S/CH	15 min, 120°C	0.05	1.08	1.27
H/CH	60 min, 150°C	0.71	10.01	44.16

[a]The abbreviations of steroids are explained in Experimental part. Chemicals.

Further research is in progress concerning the elaboration of chromatographic conditions for the separation of androsterone from dehydro-epiandrosterone and testosterone from estradiol.

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